GENETIC, ENVIRONMENTAL, AND PHYSIOLOGICAL FACTORS INFLUENCING BLOSSOM-END SCAR SIZE IN TOMATO

By

J.H.M. BARTEN

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Dedicated to my parents,
Dini and Peter Barten-van Duynhoven,
and my best friend Elizabeth
in appreciation of their
love and understanding

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TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS iii

ABSTRACT viii

CHAPTERS

1 INTRODUCTION 1

2 LOW TEMPERATURES INDUCE ROUGH BLOSSOM-END SCARRING OF TOMATO FRUIT DURING EARLY FLOWER DEVELOPMENT 5

Introduction 5
Materials and Methods 6
Results 11
Discussion 22

3 THE INFLUENCE OF GIBBERELLIC ACID AND 4-CHLOROPHENOXYACETIC ACID TREATMENT OF YOUNG FLOWERING PLANTS ON BLOSSOM-END SCAR SIZE OF TOMATO FRUIT 27

Introduction 27
Materials and Methods 29
Results 32
Discussion 44

4 DIALLEL ANALYSIS OVER TWO LOCATIONS FOR BLOSSOM-END SCAR SIZE IN TOMATO 50

Introduction 50
Materials and Methods 52
Results 54
  Griffing Analysis 54
  Hayman Analysis 59
  Allard Analysis 66
Discussion 68

5 THE INHERITANCE OF INTRA-PLANT STABILITY FOR BLOSSOM-END SCAR SIZE, CHANNELS, AND FRUIT SHAPE IN TOMATO OVER TWO ENVIRONMENTS 71

Introduction 71
Materials and Methods 72
Results 76
Generation Means Analysis Including Segregating Generations 76
Generation Means Analysis of Nonsegregating Generations 83
Intra-plant Stability and Marketability 88
Fruit Shape and Disorders 91
Discussion 95

6 THE CHARACTERIZATION OF BLOSSOM-END MORPHOLOGY GENES IN TOMATO AND THEIR USEFULNESS IN BREEDING FOR SMOOTH BLOSSOM-END SCARS 100

Introduction 100
Materials and Methods 102
Genetic Material 102
Experimental Procedure 105
Results 107
Discussion 123

7 SUMMARY AND SUGGESTIONS FOR FURTHER RESEARCH 128

APPENDICES

A THE RELATIONSHIP BETWEEN BLOSSOM-END SCAR SIZE AND NUMBER OF LOCULES 135

B THE RELATIONSHIP BETWEEN BLOSSOM-END SCAR SIZE AND SEED SET 138

The Influence of Total Seed Number and Longitudinal Distribution of Seeds Within the Fruit on Blossom-end Scar Size 138
The Effect of Pollen Dilution on Blossom-end Scar Size 142

C THE RELATIONSHIP BETWEEN BLOSSOM-END SCAR SIZE AND REGULARITY OF FRUIT SHAPE AND SEED DISTRIBUTION 147

D GENETIC AND ENVIRONMENTAL VARIATION IN BLOSSOM-END SCAR SIZE DURING EARLY AND LATE HARVESTS 151

E A THEORETICAL CONSIDERATION OF GENERATION MEANS ANALYSIS OVER TWO OR MORE ENVIRONMENTS AND TWO NEW SPREADSHEETS, ENVNOSEG AND ENVSEG, FOR ANALYSIS BY MICROCOMPUTER 156

- Introduction 156
- Generation Means Analysis Over Two Environments 158
  - Nonsegregating Generations 158
  - Segregating Generations 162
- Generation Means Analysis Over More Environments . . . 168

REFERENCES 176

BIOGRAPHICAL SKETCH 184

Abstract of Dissertation Presented to the Graduate School
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GENETIC, ENVIRONMENTAL, AND PHYSIOLOGICAL FACTORS INFLUENCING BLOSSOM-END SCAR SIZE IN TOMATO

By

J.H.M. Barten

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Major Department: Horticultural Science

In three experiments, short term low temperature treatments were applied to 'Horizon', 'Walter', and 'Solar Set' plants at the six leaf or early generative stage. The blossom-end scar index (BSI), measuring scar size, relative to fruit size, was recorded for mature fruit. Six days at 18C/10C (day/night) induced blossom-end scarring in developing ovaries 26-19 days before anthesis. This scheme was proposed as a standard method to induce rough blossom-end scarring.

Gibberellic acid (GA_3) and 4-chlorophenoxyacetic acid (4-CPA) affected 'Solar Set' flowers 9-4 days before anthesis, and 'Horizon' flowers 4 days before to 1 day after anthesis. For 'Solar Set', GA_3 increased BSI over the control and 4-CPA treatments, whereas for 'Horizon', GA_3 increased BSI over the 4-CPA, but not over the control treatment. High BSI values correlated with slow initial fruit growth. The number of locules or seeds per fruit did not influence BSI.

Diallel analysis in Bradenton, Florida, and Hazeva, Israel, indicated primarily additive inheritance of BSI. Epistatic effects were insignificant. Dominance was significant but on the average incomplete and generally in the direction of lower BSI. Heritability estimates were 0.61 and 0.70 in Bradenton and Hazeva, respectively. The significant genotype x environment interaction was due to recessive gene action in 'Suncoast' in Hazeva, which was absent in Bradenton. NC 140 and NC 8276 had low BSI values in both locations.

Generation means analysis in Bradenton and Fletcher, North Carolina, indicated that intra-plant stability for BSI inherited additively. Heritability estimates were only 0.09 and 0.38 in Bradenton and Fletcher, respectively. Intra-plant stability was relatively unimportant for marketability. For stable hybrid performance, selection against high intra-plant variation is necessary.

Another approach to breeding for smooth blossom-end scars involves the use of blossom-end morphology genes. In a complementation study, three different, undescribed nipple tip genes were identified (*n*-2, *n*-3, *n*-4). These genes were not associated with genetic leaf curl and were expressed primarily in young fruits. Intercrossing parents with different nipple tip genes seems a promising approach for breeding hybrids with smooth blossom-end scars.

CHAPTER 1
INTRODUCTION

Ideally, the stylar or blossom-end scar on tomato (*Lycopersicon esculentum* Mill.) fruit is closed and small. Frequently, however, corky scar tissue develops on the blossom-end, usually containing channels that lead to internal locular tissue. Rough blossom-end scarring (RBS) is common wherever large fruited tomatoes are grown. Fortney (1958) pointed out the presence of post-harvest pathogens in channeled fruits, causing internal discoloration. Other problems associated with rough blossom-end scarring are poor appearance, susceptibility to bruising at the blossom-end, leaking of fruit juices, and reduced shelf life (Barksdale et al., 1972; Jones et al., 1969; Young and MacArthur, 1947). Subjective observations in fresh market tomato packing houses and yield trials over many years identified RBS as a major recurring reason for rejection. Although the importance of the problem is generally recognized, no serious effort has been undertaken to investigate the physiological backgrounds or genetic regulation of the disorder. Reasons for this lack of information probably were unpredictable occurrence in field situations and absence of a reliable induction treatment.

The first purpose of this study was to identify environmental factors that cause RBS, and to develop an induction method that produces RBS with high reliability. Knavel and Mohr (1969) reported that long term (5 weeks) low temperatures during seedling growth induced catfacing; severe RBS combined with distorted fruit shape. A RBS inducing treatment based on short term low temperature exposure of plants at the six leaf or early generative stage is described in Chapter 2. Wien and Zhang (1991) reported the induction of RBS in early fruits by gibberellic acid (GA_3) foliar sprays of transplants. Chapter 3 reports on a concurrent experiment investigating the effectiveness of a GA_3 spray of young generative plants for RBS induction, and the ability of a 4-chlorophenoxyacetic acid spray to counteract the effect of low temperatures on blossom-end scar size under field conditions. Sikes and Coffey (1976) reported that delay of pruning until after initial fruit set resulted in lower occurrence of catfacing than in early pruning systems. Induction of RBS by means of pruning or topping was not pursued, since these methods dramatically change the plant architecture and would thus be unsuitable as genotype screening technique.

In this work an attempt was also made to elucidate physiological backgrounds of the disorder. Several theories have been developed on the cause of RBS although no causal relationship has yet been demonstrated. Knavel and Mohr (1969) as well as Sikes and Coffey (1976) suggested that inhibition of synthesis or transport of growth substances essential for cell division processes at the base of the style may prevent complete closure of the carpel walls, leading to RBS in mature fruits. It has also been suggested that low seed set causes RBS due to reduced auxin activity in developing ovaries. Another hypothesis is that RBS is related to irregularity of fruit shape caused by incomplete pollination in part of the fruit (Sikes and Coffey, 1976). Wien and Zhang (1991) reported a significant correlation between the number of locules and blossom-end scar size, and hypothesized that mechanical stress during growth of multilocular fruits caused problems with carpel closure. Chapters 2 and 3, and Appendices A, B, and C deal with several of these hypotheses.

The second purpose of this study was to investigate the genetics of blossom-end scar size. Detailed information on genetic regulation of blossom-end scar size and the stability of genetic parameters over environments was obtained from a diallel experiment in two locations (Bradenton, Florida, and Hazeva, Israel), reported in Chapter 4. The research described in Chapters 2, 3, and 4 was part of a United States-Israel cooperative research project on environmental effects and genetic improvement of blossom-end scarring in tomato.

The third and final purpose was to identify and characterize sources of resistance to RBS. Two different approaches were investigated. The first approach involved the use of genotypes with a high intra-plant stability for blossom-end scar size. Under RBS inducing conditions these genotypes seemed to maintain an acceptable degree of smoothness. The inheritance of intra-plant stability was investigated using a generation means analysis over two locations (Bradenton, Florida, and Fletcher, North Carolina), reported in Chapter 5. The usefulness of this approach in breeding for smooth blossom-end scarring is discussed.

The second approach involved the use of genes influencing blossom-end morphology. Tomato breeders have frequently used genotypes with a pointed blossom-end morphology to obtain smoothness. However, this pointed blossom-end character is associated with genetic leaf curl, which can increase foliar disease problems. Chapter 6 describes alternative blossom-end morphology genes without associated leaf curl. Tests for allelism of these genes with each other and with previously described blossom-end morphology genes are reported in Chapter 6. The application of these alternative genes in breeding for smooth blossom-end scarring is also discussed.

CHAPTER 2
LOW TEMPERATURES INDUCE ROUGH BLOSSOM-END SCARRING OF TOMATO FRUIT DURING EARLY FLOWER DEVELOPMENT

Introduction

Rough blossom-end scarring (RBS) in tomato (*Lycopersicon esculentum* Mill.) is characterized by corky tissue at the distal end of the fruit which may contain channels, usually extending into the locules (Fortney, 1958; Sherman and Allen, 1981). RBS is aesthetically undesirable, but more importantly, the blossom-end is sensitive to bruising. Furthermore, channels often leak and form an entrance port for post-harvest pathogens, dramatically reducing shelf-life (Fortney, 1958; Jones et al., 1969). Catfacing is a severe form of RBS, usually combining large blossom-end scars with a distorted blossom-end and irregular fruit shape (Stevenson and Heimann, 1981). Although many modern cultivars are resistant to catfacing, RBS still is one of the main reasons for rejection in the fresh market tomato industry.

The occurrence of catfacing has been associated with prolonged cool weather early in the growing season (Knavel and Mohr, 1969; Sherman and Allen, 1981; Stevenson and Heimann, 1981). In addition to catfacing, long term (≥ 5 weeks) low temperature treatments, applied between cotyledon expansion and anthesis, caused faciation (Sawhney and Polowick, 1985), triangular fruits (Hosoki et al., 1985), navel-like scars, puffiness, seedless fruit (Rylski, 1979), and strawberry fruits with split ovary (Asahira et al., 1982). Most of these malformations were attributed to an increase in the number of locules (Asahira et al., 1982; Kaneme and Itagi, 1966; Sawhney and Polowick, 1985). Since each of these experiments involved long term low temperature treatments, many fruits were affected and detailed information was not obtained on the stage of flower development sensitive to low temperature induction of fruit malformations. Kaneme and Itagi (1966) suggested that fruit malformations could only be induced when low temperatures were applied shortly before or after flower differentiation. Saito and Ito (1971) reported that a low temperature treatment of 20 days at 9C, starting at the four leaf stage, resulted in increased locule numbers in the first inflorescence. Rylski (1979) indicated that low temperatures (18C/9C day/night) induced RBS before, but not after anthesis.

The purpose of this study was to determine the effect of short term low temperatures on RBS induction, and to obtain detailed information on the growth stage during flower development that was most sensitive to inducing low temperature treatments.

Materials and Methods

Flowering plants (second inflorescence), spring 1989. 'Horizon' and 'Walter', both susceptible to RBS, were seeded on 19 January and transplanted to trays with 45 ml cells on 27 January. The plants were potted in 1.6 liter pots on 10 March and grown in a greenhouse until one to two flowers of the second inflorescence were at anthesis. On 6 April the plants were moved into growth chambers and treated as follows:

1) Control; 6 days at 22C/17C (day/night).
2) 1 day at 22C/17C, 1 day at 10C/10C, 4 days at 22C/17C.
3) 1 day at 22C/17C, 3 days at 10C/10C, 2 days at 22C/17C.
4) 1 day at 22C/17C, 5 days at 10C/10C.

The plants were illuminated for 12 hours a day with gro-lux and cool white tubes at an average photosynthetic photon flux of 170 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Twelve plants of each cultivar per treatment were planted in the field on 12 April. Flowers were tagged at anthesis so that the growth stage of each flower at the beginning of the temperature treatments could be derived in days, relative to anthesis. The flowers were tagged from 1 April until 30 April. Mature fruits were harvested and the blossom-end scar size was measured, relative to fruit size as follows:

$$BSI = \frac{\sqrt{a \times b}}{\sqrt{c \times d}} \times 100$$

with BSI = blossom-end scar index,

a = largest diameter of blossom-end scar,

b = smallest diameter of blossom-end scar,

c = largest diameter of equatorial fruit cross section,

d = smallest diameter of equatorial fruit cross section.

Examples of different levels of severity of RBS and associated BSI values are provided in Figure 2-1. Measurements of scar and fruit size were taken using an electronic caliper (Ultra-Cal, accuracy ± 0.03 mm; Fowler Sylvac) with a portable interface and datalogger ("1x8" Gage

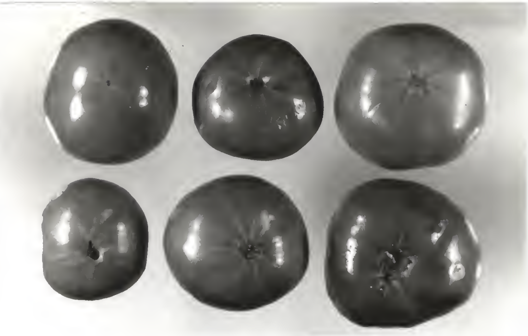

Figure 2-1. Varying degrees of rough blossom-end scarring; the values for blossom-end scar index from top left to bottom right are 5.6, 10.2, 12.6, 16.8, 18.5, and 26.6, respectively.

Talker plus; Fowler), and downloaded to a microcomputer for data analysis. The number of locules and anthesis date were also recorded.

Flowering plants (first inflorescence), spring 1990. The recently released heat-tolerant cultivar Solar Set (Scott et al., 1989) was seeded on 5 January, transplanted to trays with 45 ml cells on 17 January, potted in 0.35 liter pots on 3 February and transplanted to 1.6 liter pots on 16 March. The plants were grown in a greenhouse until one flower on the first inflorescence was at, or close to, anthesis. Starting on 28 March, the following treatments were applied to 16 plants per treatment:

1) Greenhouse control; 12 days at ambient temperatures (avg. max: 32.5C, avg. min: 18.5C) and daylight in greenhouse.
2) Growth chamber control; 12 days at 29C/18C in growth chamber.
3) 6 days at 18C/10C, 6 days at 29C/18C in growth chamber.
4) 9 days at 18C/10C, 3 days at ambient temperatures in growth chamber.

The low temperature treatments were milder than in 1989 and similar to field temperatures during a cold spell in Florida. A growth chamber malfunction on 6 April interrupted the last treatment which was going to be 12 days at 18C/10C. The illumination in the growth chambers was the same as in 1989. Flowers were tagged from 27 March until 30 April and on 9 April the plants were transplanted to greenhouse beds. BSI and anthesis date were recorded for mature fruits.

Six leaf stage, fall 1990. 'Solar Set' was seeded on 30 July, transplanted to trays with 45 ml cell volume on 8 August, and potted in 0.35 liter pots on 19 August. The plants were kept in a greenhouse until the sixth true leaf was developed. On 26 September plants were moved into the growth chamber and low temperature treatments were applied to 29 plants per treatment as follows:

1) Control; 12 days at 29C/18C.
2) 6 days at 18C/10C, 6 days at 29C/18C.
3) 9 days at 18C/10C, 3 days at 29C/18C.
4) 12 days at 18C/10C.

Illumination in the growth chamber was as described before. The plants were planted in greenhouse beds on 8 October and flowers were tagged from 21 October until 5 November. BSI and anthesis date were recorded at maturity. This large scale induction experiment was conducted to get more fruits per individual anthesis dates.

Experimental design and data analysis. Greenhouse and field experiments were conducted in Bradenton, Florida. The plants were planted in a completely randomized design (CRD) and data for individual fruits were analyzed following an unbalanced CRD. From the anthesis date and the starting date of the temperature treatments, the growth stage at the beginning of the temperature treatments was determined in days relative to anthesis for individual fruits. In 1989, each growth stage consisted of a 3-day interval, whereas in the spring of 1990, 5-day intervals were used, because in the latter experiment fewer fruits were available for a wider range of anthesis dates. The experiment in the fall of 1990 was big enough to analyze individual anthesis dates. In the spring of 1989, the model used was

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \epsilon_{ijkl} \quad \text{(I)}$$

with Y_{ijkl} = BSI of fruit l from cultivar k with temperature treatment i and growth stage j; μ = mean; α_i = temperature treatment effect; β_j = growth stage effect; γ_k = cultivar effect; $\alpha\beta_{ij}$, $\alpha\gamma_{ik}$, $\beta\gamma_{jk}$, and $\alpha\beta\gamma_{ijk}$ = interaction effects; and ϵ_{ijkl} = error. In the 1990 experiments, the model was

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ijk} \quad \text{(II)}$$

with Y_{ijk} = BSI of fruit k with temperature treatment i and growth stage j. The other symbols are as indicated for model I. PROC GLM of Statistical Analysis System was used for analysis of variance (ANOVA) and the LSMEANS statement was used to obtain least squares means. Pairwise comparisons between temperature treatments at fixed levels of growth stage and cultivar effects were made using CONTRAST statements in PROC GLM (Freund et al., 1986). Multiple range tests were constructed from pairwise comparisons with α/n as the level of significance for individual pairwise comparisons, where α is the level of significance for the multiple range test and n is the number of pairwise comparisons possible.

Results

In all experiments, 4 to 5 days after the start of the low temperature treatments, anthocyanin had accumulated in the veins and stems of the plants, and the leaves were dark green. The control plants were taller than the other treatments. Two weeks after the end of the treatments, no difference in plant appearance was noticeable between treatments. The low temperature treatments did not influence the average number of fruits harvested per plant (Tables 2-1 and 2-2). Of the fruits in each experiment, 0% to 1% was fasciated regardless of treatment and eliminated from the analysis. The occurrence of beaky (i.e. exhibiting pointed blossom-ends) and puffy fruits on some of the plants in the greenhouse was unrelated to the cold treatments and these fruits were included in the analysis.

Flowering plants (second inflorescence), spring 1989. In this experiment there was no difference in earliness, expressed as days from seeding to anthesis, between 'Horizon' and 'Walter'. ANOVA according to model I indicated significant temperature treatment x growth stage interaction (Table 2-3), suggesting that the low temperature treatments

Table 2-1. Number of plants, fruits and average number of fruits per plant used in the analysis of the temperature induction experiment in 1989.

Treatment	Horizon			Walter		
	Plants (no.)	Fruits (no.)	Fruits/ plant	Plants (no.)	Fruits (no.)	Fruits/ plant
Control	12	175	14.6	12	180	15.0
1 day 10C	12	180	15.0	12	153	12.8
3 days 10C	12	168	14.0	12	162	13.5
5 days 10C	12	177	14.8	12	164	13.7

Table 2-2. Number of plants, fruits and average number of fruits per plant used in the analysis of temperature induction experiments with 'Solar Set' in 1990.

Treatment	Flowering plants Spring 1990			Six leaf stage Fall 1990		
	Plants (no.)	Fruits (no.)	Fruits/ plant	Plants (no.)	Fruits (no.)	Fruits/ plant
Greenhouse control	16	140	8.8	-	-	-
Growth chamber control	16	107	6.7	29	182	6.3
6 days 18C/10C	16	138	8.6	29	215	7.4
9 days 18C/10C	16	138	8.6	29	190	6.6
12 days 18C/10C	-	-	-	29	196	6.8

only affected flowers at a particular growth stage. Significance of the growth stage x cultivar interaction (Table 2-3) may indicate a difference between 'Horizon' and 'Walter' in sensitivity to the low temperature treatments. For each combination of temperature treatment, growth stage, and cultivar, the least squares means for BSI and number of locules were calculated, and plotted in Figure 2-2. In Figure 2-2 the growth stage at the beginning of the cold treatment is given in days

Table 2-3. Analysis of variance for blossom-end scar index (BSI) and number of locules for the temperature induction experiment in the spring of 1989.

Source	BSI			Number of locules		
	df	MS	*P* value	df	MS	*P* value
Temperature treatment	3	12.28	0.496	3	1.25	0.530
Growth stage	9	422.59	<0.001	9	43.54	<0.001
Cultivar	1	74.38	0.028	1	575.29	<0.001
Treatment x growth stage	27	33.09	<0.001	27	2.59	0.043
Treatment x cultivar	3	5.48	0.786	3	0.22	0.942
Growth stage x cultivar	9	49.60	<0.001	9	6.72	<0.001
Treatment x stage x cultivar	27	8.63	0.967	27	2.14	0.169
Error	1278	15.43		1265	1.70	
Total	1357			1344		

relative to anthesis, with negative and positive values indicating days before and after anthesis, respectively. Thus, the position on the X-axis indicates the age of the fruits, with the youngest fruits closest to the origin. Per cultivar and growth stage, multiple range tests were constructed from pairwise comparisons between temperature treatments as described before (Figure 2-2). For 'Horizon' fruits that were 23-21 days before anthesis at the beginning of the cold treatment, 5 days at 10C induced significantly larger blossom-end scars as compared to the other treatments (Figure 2-2A). 'Walter' reacted similarly to cold exposure, although 3 days at 10C had an intermediate effect (Figure 2-2C). 'Horizon' and 'Walter' fruits that were 4-6 days past anthesis at the start of the cold treatment (7 April) had increased BSI for all treatments (Figures 2-2A and 2-2C). These fruits were at anthesis on 1-3 April and 25-23 days before anthesis at the beginning of a cold spell that lasted from 9-12 March (Figure 2-3). This cold spell probably induced RBS in all plants, regardless of low temperature treatments applied later. Thus, in this experiment two lines of evidence indicated that flower buds were most sensitive to low temperatures 25-21 days before anthesis. At this growth stage, flower initials are not visible without magnification. Five days at 10C induced increased RBS without causing severe low temperature related disorders.

The pattern for average number of locules per fruit in Figures 2-2B and 2-2D deviated markedly from that for BSI in Figures 2-2A and 2-2C. The average number of locules in 'Horizon' fruit was not affected by the low temperature treatments (Figure 2-2B). The average number of

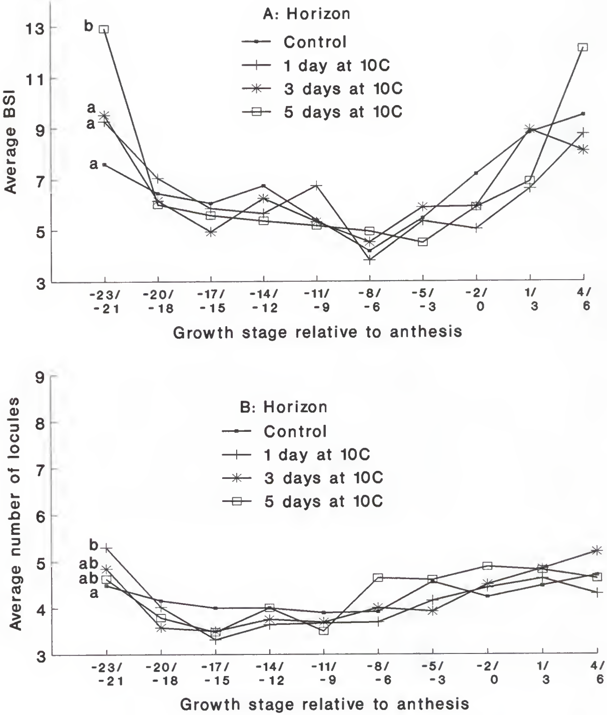

Figure 2-2. The influence of low temperature treatments on 'Horizon' (A and B) and 'Walter' (C and D) flowers at different growth stages, expressed in days relative to anthesis, with negative and positive values indicating days before and after anthesis, respectively. Treatments with a different letter within a growth stage are significantly different at $\alpha = 0.05$. Each data point represents an average of at least five fruits.

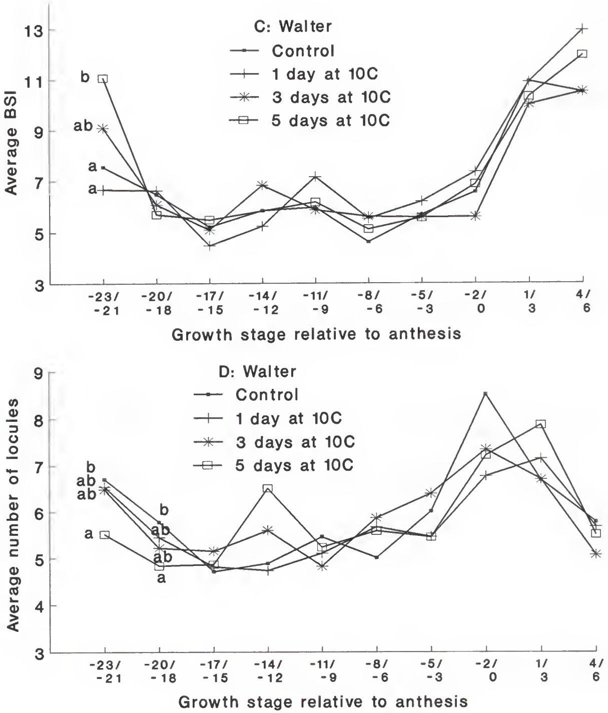

Figure 2-2. Continued.

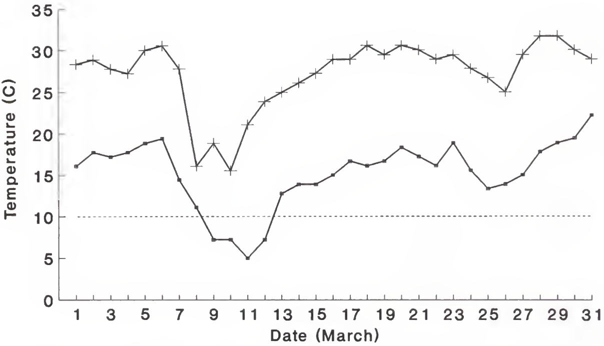

Figure 2-3. Minimum (▪) and maximum (+) daily temperatures for March, 1989, in the greenhouse where 'Walter' and 'Horizon' were grown before temperature treatments were applied to flowering plants.

locules was high for all treatments in 'Walter' fruits that were 2 days before to 3 days after anthesis at the start of the cold treatment (Figure 2-2D). These fruits were at anthesis on 4-9 April and 31-26 days before anthesis at the beginning of the cold spell in March, which may have caused this effect. Increased average BSI values were not associated with increased average locule numbers, indicating that increased locule numbers were not a direct cause of RBS in this experiment.

Flowering plants (first inflorescence), spring 1990. This experiment was designed to further investigate the significant temperature effects at the edge of the graphs in Figures 2-2A and 2-2C.

Table 2-4. Analysis of variance for blossom-end scar index for the temperature induction experiments in 1990.

Source	Flowering plants (spring 1990)			Six leaf stage (fall 1990)		
	df	MS	*P* value	df	MS	*P* value
Temperature treatment	3	1.93	0.817	3	38.03	<0.001
Growth stage	6	34.26	<0.001	15	14.65	<0.001
Treatment x growth stage	18	14.34	0.002	41	7.60	<0.001
Error	495	6.19		723	2.27	
Total	522			782		

No confounding climatic conditions occurred before the temperature treatments. ANOVA for BSI according to model II indicated significant temperature treatment x growth stage interaction (Table 2-4), suggesting sensitivity of a particular growth stage to low temperatures. For each combination of temperature treatment and growth stage, the least squares means were calculated for BSI and plotted in Figure 2-4. Per growth stage, multiple range tests were constructed for the temperature treatments as described before (Figure 2-4). Only flowers that were 23-19 days before anthesis at the start of the low temperature treatments were sensitive to the low temperature treatments (Figure 2-4). For fruits that were 23-19 days before anthesis at the start

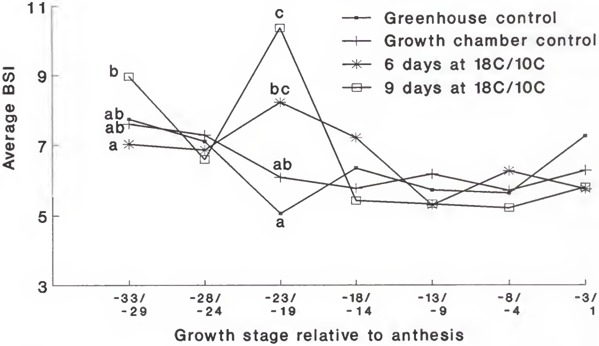

Figure 2-4. The influence of low temperature treatments on 'Solar Set' flowers at different growth stages, expressed in days relative to anthesis, with negative and positive values indicating days before and after anthesis, respectively. Treatments with a different letter within a growth stage are significantly different at $\alpha = 0.05$. Each data point represents an average of at least five fruits.

of the treatments, the average BSI did not differ significantly between the two control treatments, indicating that the transfer of plants from greenhouse to growth chamber did not influence blossom-end scarring. Nine days at 18C/10C caused significantly increased values for BSI as compared to the control treatments, whereas 6 days cold treatment had an intermediate effect on flowers that were 23-19 days before anthesis (Figure 2-4). Although fruits that were 33-29 days before anthesis had a high average BSI value for the 9-day cold treatment, this value was not significantly different from the BSI values for the two control treatments.

Six leaf stage, fall 1990. ANOVA according to model II indicated significant temperature treatment x growth stage interaction (Table 2-4). Least squares means were calculated for BSI per combination of growth stage and temperature treatment, and multiple range tests were constructed for the temperature treatments within each growth stage (Figure 2-5). The 9- and 12-day cold treatment flowered 3 and 2 days later than the other treatments, respectively (Figure 2-5). Unfortunately, flower bud differentiation was delayed for all treatments and at least two extra leaves developed between the cotyledons and the first inflorescence, probably due to high temperatures shortly after cotyledon expansion (Calvert, 1957). Thus, the oldest flowers were 25 days before anthesis at the beginning of the cold treatments. Although the flowers were younger than anticipated at the beginning of the temperature treatments, the data in Figure 2-5 were in agreement with the previous experiments. Six days at 18C/10C resulted in significantly increased levels of BSI over the control treatment for fruits that were

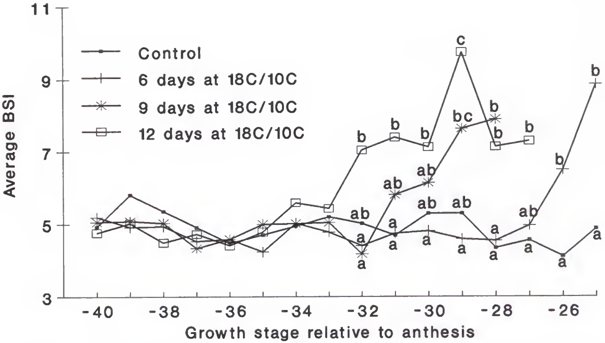

Figure 2-5. The influence of low temperature treatments on 'Solar Set' flowers at different growth stages, expressed in days relative to anthesis, with negative and positive values indicating days before and after anthesis, respectively. Treatments with a different letter within a growth stage are significantly different at $\alpha = 0.05$. Each data point represents an average of at least five fruits.

25 and 26 days before anthesis at the beginning of the treatments (Figure 2-5). Nine days at 18C/10C induced significantly increased levels of BSI over the control treatment for fruits that were 28 days before anthesis at the beginning of the treatment (Figure 2-5). Thus flowers that were sensitive to the 9-day treatment were 2 days younger at the beginning of the treatment than the flowers that were sensitive to the 6-day treatment. A possible explanation is that these younger flowers grew into the sensitive stage (26 days before anthesis) during the 9-day treatment and subsequently received 6 days at 18C/10C. Similarly, 12 days at 18C/10C induced significantly increased levels of

BSI in flowers that were 32 days before anthesis or older at the beginning of the treatment (Figure 2-5). This means, that flowers that were sensitive to the 12-day treatment were 6 days younger at the beginning of the treatment than those that were sensitive to the 6-day treatment. This fits the hypothesis that the younger flowers grew into the sensitive stage (26 days before anthesis) during the 12-day treatment and subsequently received 6 days at 18C/10C. If flowers were sensitive to 6 days at 18C/10C earlier than 26 days before anthesis, then the 6-day treatment should have affected these earlier stages as well. The conclusion from this experiment was that 6 days at 18C/10C was enough to induce RBS and that flowers were sensitive to RBS inducing temperatures no earlier than 26 days before anthesis. The intermediate effect of 6 days at 18C/10C on flowers 23-19 days before anthesis in the spring of 1990 (Figure 2-4) may have been due to pooling of anthesis dates.

Discussion

In each experiment, flowers were sensitive to low temperature induced RBS for only a short period during their development. Five days at 10C or 6 days at 18C/10C were sufficient to induce RBS. These results explain the variation in expression of RBS over seasons and within plants often occurring in field situations (Elkind et al., 1990b). The long time interval between induction and visible response explains why it has been hard to relate the occurrence of RBS to temperature fluctuations. For the fruits that underwent inducing temperatures and developed in the field, the percentage unmarketable fruits (scar length > 1 cm; Wien and Zhang, 1991) was 35% and 31% in 'Horizon' and 'Walter', respectively (1989 experiment). Thus, if climatic conditions are inductive, losses due to RBS can be quite extensive. The sensitive period was 26-19 days before anthesis and very similar in experiments involving three different growing seasons, three different cultivars, grown in field or greenhouse beds. This information could be used in field situations to investigate whether genotypes are sensitive to low temperature induction of RBS. Three weeks after a cold spell, flowers can be tagged at anthesis and blossom-end scar size on mature tagged fruits can then be evaluated for different genotypes. This method of focusing observations during selection will help breeding for smooth blossom-end scars. Recent genetic studies indicated predominantly additive inheritance of blossom-end scar size (Barten et al., 1991b) and significant, but relatively unimportant genotype x environment interactions (Barten et al., 1991b; Elkind et al., 1990b).

This study presents a standard induction technique for further physiological studies or genotype screening. A short, mild, low temperature treatment (6 days at 18C/10C) will induce RBS without affecting plant development (Knavel and Mohr, 1969; Sawhney and Polowick, 1985), reducing fruit set (Wien and Zhang, 1991), or causing severe temperature related fruit malformations. Temperature treatments are effective only after flowers have been initiated, usually at the three to four leaf stage (Hurd and Cooper; 1970). After cold exposure plants can be grown in greenhouse beds or the field. When plants were grown in pots in a greenhouse after temperature induction, BSI did not increase over control treatments (data not shown). Apparently RBS induction treatments are more effective in a post-treatment environment that is favorable for vigorous growth and development. Research by Abel and Kedar (1987, unpublished results) indicated that low temperature induction treatments may be helpful in genotype screening. They reported that low temperature treatment (17C/12C) from cotyledon expansion until flowering induced significant differences in BSI between five genotypes, indicative of their performance in field situations. In the induction experiment of fall 1990, 9 days at 18C/10C did not result in increased BSI values for fruits that were at the sensitive stage in the resistant cultivar Valerie (data not shown due to low fruit numbers).

Studies of tomato flower ontogeny indicated that 26 days before anthesis sepals are differentiated and the corolla starts developing (Chandra Sekhar and Sawhney, 1984; Saito and Ito, 1971; Smith, 1935). At 19 days before anthesis stamens and carpels start to differentiate. Thus, low temperatures induce RBS very early during differentiation of the ovary. Knavel and Mohr (1969), studying severe catfacing induced by a 5-week low temperature (13C/6C) treatment during seedling growth, reported breakdown of tissue at the stylar base sometime prior to anthesis. Further studies are needed to characterize initiation of RBS at the cellular level. Previous studies suggested that the number of locules per fruit is determined shortly after flower bud initiation (Kaneme and Itagi, 1966; Saito and Ito, 1971). In the present study, low temperatures induced an increase in locule numbers in 'Walter' flowers during initial flower differentiation (31-26 days before anthesis). It seems that during flower differentiation, the number of locules is determined prior to blossom-end scar size; however, more work is needed to further substantiate this hypothesis.

The small changes in locule numbers observed in the present study (Figure 2-2) are in strong contrast with the results of Saito and Ito (1971), who reported that 6 days at 17C/8C induced doubling of the average number of locules per fruit in their material. Multilocular genotypes, with 8-16 locules, like those used by Saito and Ito (1971), apparently were more sensitive to low temperature induced locule proliferation than genotypes with only a few (3-7) locules per fruit (Asahira et al., 1982; Rylski, 1979; Sawhney and Polowick, 1985). In the present experiment, increased average values for BSI were not associated with increased average locule numbers in the same fruit (Figure 2-2). These results agree with a related study in which application of gibberellic acid (GA_3) and 4-chlorophenoxyacetic acid to young, generative plants affected average BSI, but not the average number of locules. Furthermore, investigation of an array of 17 cultigens, varying in sensitivity to RBS induction, indicated that the average scar size per genotype was unrelated to the average number of locules ($r = 0.13$; Appendix A). Apparently, factors other than the number of locules play a role in RBS induction. However, these results do not reject the possibility that induction of locule proliferation also causes RBS due to mechanical stress during fruit growth, especially in multilocular genotypes. Wien and Zhang (1991) reported that foliar sprays of $4.3 \cdot 10^{-5}$ M GA_3 twice weekly for 3 weeks after transplanting, in multilocular genotypes. Wien and Zhang (1991) reported that foliar sprays of $4.3 \cdot 10^{-5}$ M GA_3 twice weekly for 3 weeks after transplanting, low temperatures (21C/10C day/night) for 3 weeks after transplanting, or topping at transplanting induced locule proliferation and RBS in the multilocular cultivar Celebrity (8-10 locules). Wien and Zhang (1991) started their treatments between the two and three leaf stage and RBS was induced in fruits of the first three clusters. Wien and Turner (1991) reported that 2 weeks at 16C/10C (day/night), starting at the six leaf stage, induced RBS in fruits of the second and third, but not the first cluster. Although in this work anthesis dates were not recorded, the sensitive period during flower development seems to precede the developmental stage sensitive to low temperature RBS induction in the present experiment. It is possible, that locule proliferation causes RBS in multilocular genotypes, explaining sensitivity during early stages of flower differentiation. Alternatively, low temperature treatments, lasting for 3 weeks or longer, may induce locule proliferation as well as RBS, although the sensitive period during flower differentiation is not the same for these two processes.

CHAPTER 3
THE INFLUENCE OF GIBBERELLIC ACID AND 4-CHLOROPHENOXYACETIC ACID TREATMENT OF YOUNG FLOWERING PLANTS ON BLOSSOM-END SCAR SIZE OF TOMATO FRUIT

Introduction

Rough blossom-end scarring (RBS) in tomato (*Lycopersicon esculentum* Mill.) is characterized by enlarged scar tissue at the distal end of the fruit and is of great economic importance in the fresh market tomato industry. Large blossom-end scars often contain channels that extend into locular tissues, resulting in leaking of fruit juices, infection with post-harvest pathogens, and reduced shelf life (Jones et al., 1969; Sherman and Allen, 1981). Barten et al. (1991a) reported that short term (6-day) low temperature treatments (18C day/10C night) applied during early flower differentiation induced RBS in mature fruits. Developing ovaries in prefloral and young generative plants were most sensitive to low temperatures from 26 to 19 days before anthesis. Cool weather during early flower development was suggested to be a cause of RBS in field situations (Barten et al., 1991a; Rylski, 1979).

Gibberellic Acid (GA_3) induced catfacing, a severe form of RBS, when applied as a foliar spray at transplanting or shortly thereafter

(Courter and Drinkwater, 1961; Robbins and Hernandez, 1970). Wien and Zhang (1991) reported that sprays of $4.3 \cdot 10^{-5}$ M GA_3 twice weekly for 3 weeks after transplanting induced RBS comparable to a 3-week low temperature treatment (21C/10C). Sawhney (1983) suggested that the effect of low temperatures (18C/15C) during seedling growth on proliferation of floral organs might be caused by increased levels of endogenous GA_3. Hosoki et al. (1985) reported increased GA_3 and decreased diffusible auxin levels in shoot apices of seedlings at the four leaf stage after a long term (25 days) low temperature treatment (daily minimum below 10C) which induced severe fruit malformations (ovary split, fasciation, and oblate fruits) in early fruits. Exogenous application of 4-chlorophenoxyacetic acid (4-CPA) at the three leaf stage decreased locule numbers and occurrence of low temperature induced fruit malformations in early fruits (Asahira et al., 1982). Most of the low temperature induced fruit malformations in multilocular genotypes (8-16 locules per fruit) have been attributed to locule proliferation (Asahira et al., 1982; Hosoki et al., 1985). However, Barten et al. (1991a) reported that RBS induction was not related to increased locule numbers in genotypes with only a few (3-7) locules per fruit.

Histological studies by Knavel and Mohr (1969) indicated that 5 weeks at 13C/6C during early seedling growth caused incomplete development of the ovary wall at the stylar base, visible sometime prior to anthesis and resulting in severe catfacing in mature fruits. Sikes and Coffey (1976) reported that removal of axillary shoots during flower bud development increased the incidence of catfacing, but not when delayed until after initial fruit set. The authors of both papers speculate that inhibition of synthesis or transport of growth substances essential for cell division processes at the base of the style before anthesis may prevent complete closure of the carpels. Bünger-Kibler and Bangerth (1983) induced parthenocarpic fruit growth in emasculated ovaries with GA_3 ($1.7 \cdot 10^{-2}$ M), indole-3-acetic acid (IAA; 0.61 M), and 4-CPA ($5.4 \cdot 10^{-3}$ M) treatments and studied the influence of these growth regulators on cell division processes in the pericarp during the first 15 days of fruit development. Cell division activity in GA_3 treated ovaries was about 25% of IAA treated ovaries and seeded controls. The final cell number in 4-CPA treated fruits was higher than in IAA treated fruits and seeded controls due to a prolonged cell division period (Bünger-Kibler and Bangerth, 1983).

The purpose of the present study was to investigate the influence of GA_3 and 4-CPA, when applied to young flowering plants, on RBS induction in resistant and susceptible cultivars, and to identify the stage during flower development that was most sensitive to RBS induction. The influence of GA_3 and 4-CPA on early fruit growth, number of locules per fruit, and number of seeds per fruit was investigated in relation to blossom-end scar size.

Materials and Methods

'Horizon' (very susceptible to RBS), 'Solar Set' (moderately susceptible), and 'Valerie' (resistant) were seeded on 5 January 1990 and transplanted to trays with 45 ml cells on 17 January. The plants were set in the field at Bradenton, Florida, on 27 February in a 3x3 factorial randomized complete block design with four replications, three cultivars, three growth regulator treatments, and ten plants per plot. The plants were staked, tied, and watered by seepage irrigation. Fertilizers and pesticides were applied following recommended cultural practices. On 6 April, when most plants had at least one open flower, apices and flower clusters were sprayed with GA_3 ($2.9 \cdot 10^{-5}$ M), 4-CPA ($1.3 \cdot 10^{-4}$ M), or water. From 5 until 20 April, flowers were tagged at anthesis so that the developmental stage of each flower at the time of growth regulator treatment could be derived in days relative to anthesis.

For fruits with anthesis dates 10, 12, and 14 April, the largest and smallest diameter of the equatorial fruit cross section were measured 14 days after anthesis and the fruit size was calculated as the average of the two measurements. Fruit growth is characterized by rapid cell division in the pericarp until 14 days after anthesis, followed by mainly cell enlargement until maturity (Geelen et al., 1987; Smith, 1935). The fruit size 14 days after anthesis is an indication of cell division activity in developing ovaries (Bohner and Bangerth, 1988a,b; Bünger-Kibler and Bangerth, 1983). Mature fruits were harvested and the blossom-end scar size was measured, relative to fruit size as follows:

$$BSI = \frac{\sqrt{a \times b}}{\sqrt{c \times d}} \times 100$$

with BSI = blossom-end scar index,

a = largest diameter of blossom-end scar,

b = smallest diameter of blossom-end scar, c = largest diameter of equatorial fruit cross section,

d = smallest diameter of equatorial fruit cross section.

Measurements of scar and fruit size were taken using an electronic caliper (Cultra-Cal II, accuracy ± 0.03 mm; Fowler-Sylvac) with a portable interface and datalogger ("1x8" GageTalker plus; Fowler), and downloaded to a microcomputer for data analysis. The occurrence of channels, navel-like blossom-ends, zippers, and open locules (separation of the pericarp along a zipper scar, exposing placental tissue) was recorded for each fruit. For fruits that were at anthesis on 10 and 12 April, the number of seeds and locules were counted.

BSI values were averaged for each combination of replication, cultivar, growth treatment, and anthesis date. Analysis of variance (ANOVA) was performed on these means, with as weights the number of observations from which each mean was computed. The model was

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \beta\gamma_{jk} + \gamma\delta_{kl} + \beta\delta_{jl} + \beta\gamma\delta_{jkl} + \epsilon_{ijkl} \quad \text{(I)}$$

with Y_{ijkl} = Average BSI for fruits from replication i of cultivar j with anthesis date k and growth regulator treatment l; μ = mean; α_l = replication effect; β_j = cultivar effect; γ_k = anthesis date effect; δ_l = growth regulator effect; $\beta\gamma_{jk}$, $\gamma\delta_{kl}$, $\beta\delta_{jl}$, and $\beta\gamma\delta_{jkl}$ = interaction effects; and ϵ_{ijkl} = error. PROC GLM of Statistical Analysis System was used for weighted ANOVA and the LSMEANS statement was used to obtain least squares means. Pairwise comparisons for main effects were obtained by use of the PDIFF option in the LSMEANS statement. Pairwise comparisons between levels of one main factor at fixed levels of the other main factors were made using CONTRAST statements in PROC GLM (Freund et al., 1986). Multiple range tests were constructed from pairwise comparisons with α/n as the level of significance for individual pairwise comparisons, where α is the level of significance for the multiple range test and n is the number of pairwise comparisons possible.

For data analysis on fruit size 14 days after anthesis, number of seeds per fruit, and number of locules per fruit, model I was used for weighted ANOVA of means and multiple range tests were constructed as described before. The percentages of fruits with channels, navels, zippers, and open locules were calculated for each combination of replication, cultivar, and growth regulator treatment, resulting in a balanced data-set which was analyzed using the model

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \beta\gamma_{jk} + \epsilon_{ijk} \quad \text{(II)}$$

with Y_{ijk} = percentage fruit showing malformation in replication i of cultivar j with growth regulator treatment k; μ = mean; α_i = replication effect; β_j = cultivar effect; γ_k = growth regulator effect; $\beta\gamma_{jk}$ = interaction effect; ϵ_{ijk} = error. Sample variances were related to the mean and homogeneity of variance was obtained by transforming the data to $\log_{10}(x + 1)$ before ANOVA and Duncan's multiple range tests (Bartlett, 1947).

Results

Differences in plant appearance were noticeable several days after application of GA_3 and 4-CPA. Treatment with 4-CPA resulted in epinasty, adaxial curling of leaf margins, leaf distortion, and dark green foliage in developing shoots. GA_3 promoted shoot growth and young foliage was light green. GA_3 did not induce style elongation as described by Bukovac and Honma (1967) in any of the three determinate genotypes used in this experiment. Subjective observations on flower numbers indicated no major differences between growth regulator treatments. The average number of fruits set per plant was not significantly influenced by treatment with GA_3 or 4-CPA in any of the cultivars (data not shown). The fruit size at maturity was calculated as the average of the largest and smallest diameter of the equatorial fruit cross section. For 'Horizon' and 'Solar Set', the average mature fruit size in 4-CPA treated plots was significantly larger than in control plots, whereas GA_3 treated plots of 'Solar Set' yielded significantly smaller fruits than control plots (data not shown). For 'Valerie', only early fruits in 4-CPA treated plots were significantly larger than those from control plots, and GA_3 treatment did not affect fruit size (data not shown).

Weighted ANOVA of average BSI data according to model I indicated significant main (α = 0.05) and interaction (α = 0.10) effects (Table 3-1). For each combination of cultivar, anthesis date, and growth regulator treatment, the least squares means for BSI were calculated and plotted in Figure 3-1. Per cultivar and anthesis date, multiple range tests were constructed from pairwise comparisons between growth regulator treatments as described before (Figure 3-1). For 'Horizon' and 'Solar Set', fruits that were at anthesis from 10-16 April had high average BSI values (Figure 3-1). These high BSI values probably were induced by a 5-day cold spell from 21-25 March, with minimum night temperatures around 10C (Figure 3-2). Fruits with high levels of BSI were at anthesis from 10-16 April, and 20-26 days before anthesis on 21

Table 3-1. Analysis of variance for blossom-end scar index.

Source	df	MS	*P* value
Replication	3	210.72	<0.001
Cultivar	2	33302.47	<0.001
Anthesis date	15	1158.93	<0.001
Growth regulator treatment	2	461.53	<0.001
Cultivar x anthesis date	30	166.43	<0.001
Cultivar x treatment	4	99.37	<0.001
Anthesis date x treatment	30	30.07	0.060
Cultivar x anthesis date x treatment	60	27.02	0.071
Error	424	20.67	
Total	570		

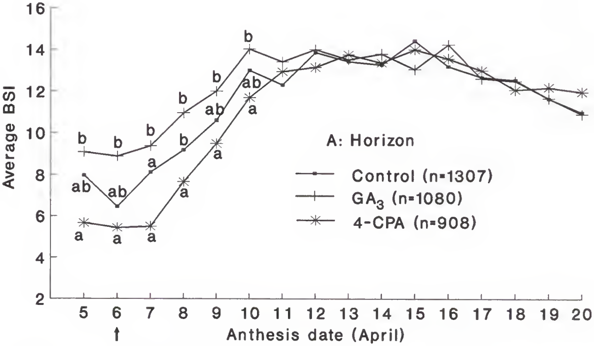

Figure 3-1. The influence of GA$_3$ and 4-CPA on flowers of 'Horizon' (A), 'Solar Set' (B), and 'Valerie' (C) at different stages of development at the time of application (6 April; ↑). Treatments with a different letter per anthesis date are significantly different at $\alpha = 0.05$. Each data point represents an average of at least 20 fruits.

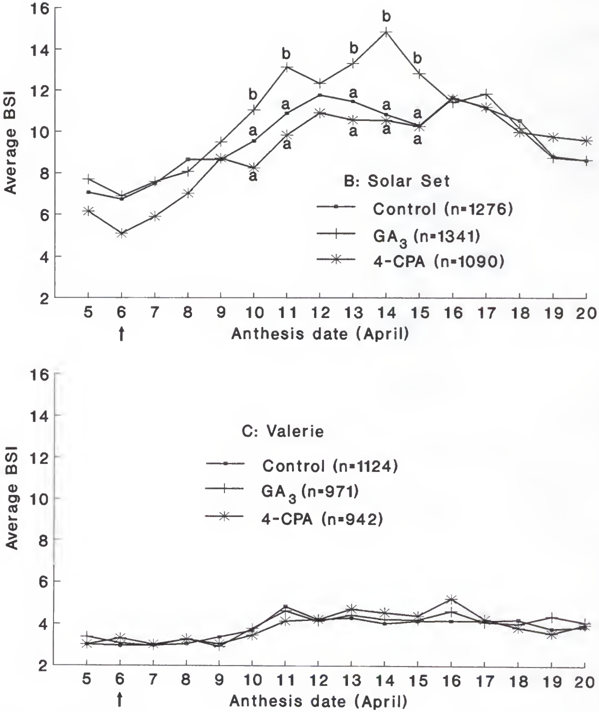

Figure 3-1. Continued.

March, at the beginning of the cold spell. This agrees with previous studies indicating that developing flower buds were most sensitive to low temperatures 19-26 days before anthesis (Barten et al., 1991a). 'Valerie' was not affected by the RBS inducing low temperatures (Figure 3-1C). For control fruits of 'Horizon' and 'Solar Set' that were at anthesis from 10-16 April, the average BSI was 13.1 and 10.8, respectively, indicating that 'Horizon' was more sensitive to RBS induction than 'Solar Set'.

For the most sensitive cultivar Horizon, growth regulators affected BSI for fruits that were at anthesis from 5-10 April, but not for those at anthesis from 11-16 April (Figure 3-1A). The maximum level of average BSI under Florida field conditions is generally around 13-14 and 'Horizon' fruits that were at anthesis from 11-16 April exhibited

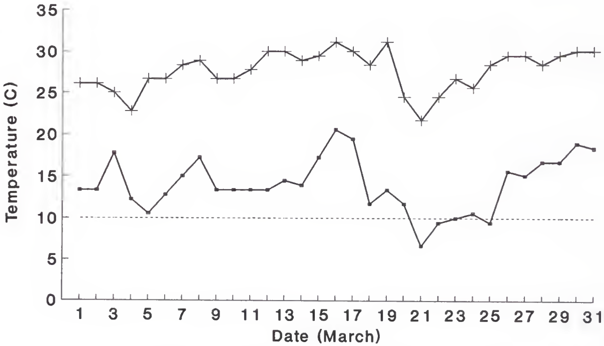

Figure 3-2. Minimum (▪) and maximum (+) daily temperatures for March 1990.

this maximal average BSI, regardless of growth regulator treatment. For 'Horizon' fruits that were at anthesis from 5-10 April, BSI values for the GA_3 treatment were significantly higher than for the 4-CPA treatment; however, neither growth regulator treatment was significantly different from the control treatment (Figure 3-1A). 'Solar Set' was less sensitive than 'Horizon' and only flowers that were at anthesis from 10-15 April, and underwent RBS inducing low temperatures during the sensitive stage of their development (20-26 days before anthesis), were affected by the growth regulator treatments (Figure 3-1B). For 'Solar Set' fruits with anthesis dates from 10-15 April, BSI values were significantly higher for the GA_3 treatment than for the control treatment, whereas BSI values for the 4-CPA and control treatments were not significantly different (Figure 3-1B). Growth regulator treatments did not significantly influence BSI for 'Solar Set' fruits that were at anthesis from 5-9 April, although BSI values seemed to be slightly reduced by the 4-CPA application (Figure 3-1B). 'Horizon' and 'Solar Set' flowers that were at anthesis on 16 April or later were not affected by the growth regulator treatments. The effect of low temperatures on BSI was more prominent than that of the growth regulators applied in this study. 'Valerie' was not affected by the growth regulator treatments (Figure 3-1C).

The data on fruit size 14 days after anthesis, number of seeds per fruit, and number of locules per fruit were used to investigate physiological backgrounds of RBS induction. Weighted ANOVA of means according to model I indicated no significant interaction effects for each of the three characteristics ($\alpha = 0.10$; Table 3-2). Least squares

Table 3-2. Analysis of variance for fruit size 14 days after anthesis, number of seeds per fruit, and number of locules per fruit.

Source	Early fruit size			Seed number			Locule number		
	df	MS	*P* value	df	MS	*P* value	df	MS	*P* value
Replication	3	25.16	0.741	3	13037	0.210	3	6.959	0.074
Cultivar	2	826.69	<0.001	2	220984	<0.001	2	127.871	<0.001
Anthesis date	2	451.04	0.001	1	102531	0.001	1	31.558	0.002
Growth regulator treatment	2	189.21	0.050	2	100669	<0.001	2	0.111	0.962
Cultivar x anthesis date	4	109.77	0.135	2	3405	0.666	2	3.564	0.292
Cultivar x treatment	4	36.60	0.659	4	1569	0.943	4	1.465	0.722
Anthesis date x treatment	4	115.98	0.116	2	7533	0.411	2	0.709	0.779
Cultivar x anthesis x treatment	8	29.37	0.862	4	5329	0.636	4	1.798	0.639
Error	71	60.36		48	8318		48	2.825	
Total	100			68			68		

means were calculated for the main effects, and multiple range tests were constructed from pairwise comparisons as described before (Table 3-3). The cultivars differed significantly for each of the characteristics. Fruits that were at anthesis on 10 April were significantly larger 14 days after anthesis than those with anthesis dates 12 and 14 April. This difference could not be explained by a higher number of seeds or locules per fruit (Table 3-3). The average temperatures during fruit development were very similar for fruits measured on 24, 26 or 28 April (Figure 3-3). Temperature differences shortly after anthesis or some other critical stage during early ovary

Table 3-3. Average fruit size 14 days after anthesis, number of seeds per fruit, and number of locules per fruit for each cultivar, anthesis date, and growth regulator treatment.

	Early fruit size[z]		Seed number		Locule number	
	Fruit no.	Average (mm)	Fruit no.	Average (no.)	Fruit no.	Average (no.)
Cultivar						
Horizon	377	32.7 a[y]	175	128 a	175	4.4 a
Solar Set	383	32.8 a	306	196 b	306	5.6 b
Valerie	319	29.9 b	190	192 b	190	6.2 c
Anthesis date						
10 April	369	33.1 a	376	157 a	376	5.2 a
12 April	392	31.0 b	295	186 b	295	5.7 b
14 April	318	31.3 b	-	-	-	-
Growth regulator treatment						
Control	402	32.3 a	250	182 a	250	5.4 a
GA_3	368	30.9 a	151	191 a	151	5.4 a
4-CPA	309	32.2 a	270	141 b	270	5.4 a

[z] Fruit size is calculated as the average of largest and smallest diameter of median fruit cross section.

[y] Values with the same letter are not significantly different in constructed multiple range test (see text) with $\alpha = 0.05$.

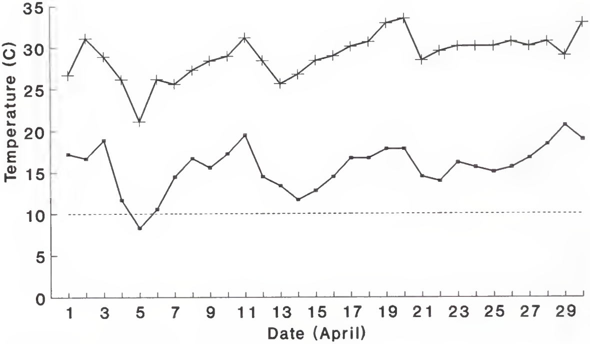

Figure 3-3. Minimum (▪) and maximum (+) daily temperatures for April 1990.

differentiation may be the cause of difference in early fruit size for different anthesis dates. The significantly higher average number of seeds per fruit for fruits that were at anthesis on 12 April as compared to those at anthesis on 10 April (Table 3-3) is probably the result of better pollination during windy weather on 12 April. Although early fruit size did not differ significantly between growth regulator treatments in a constructed multiple range test (Table 3-3), pairwise comparison indicated that the GA_3 treatment reduced initial fruit growth as compared to the control treatment ($P = 0.026$). Seed numbers were significantly reduced in 4-CPA treated fruits as compared to fruits from control and GA_3 treatments (Table 3-3). The growth regulator treatments did not affect average locule numbers in fruits that were at anthesis on 10 and 12 April (Table 3-3).

Per cultivar, the average BSI for each combination of anthesis date and growth regulator treatment was correlated with average early fruit size, average seed number per fruit, and average locule number per fruit (Table 3-4). For fruits that were at anthesis from 10-14 April, the growth regulator treatments significantly influenced BSI only in 'Solar Set' (Figure 3-1). Consequently, the correlation coefficients in Table 3-4 are most meaningful for 'Solar Set'. The Pearson correlation coefficient between BSI and early fruit size was negative and significant for 'Solar Set' (Table 3-4), indicating that slow initial fruit growth correlated with large blossom-end scars. High values for BSI correlated with high numbers of seed per fruit and this correlation was significant for 'Horizon' and 'Solar Set'. The correlation between number of locules per fruit and BSI was insignificant for 'Horizon' and 'Solar Set' (Table 3-4).

For each field plot (i.e., each combination of replication, cultivar, and growth regulator treatment), the percentages of fruits with channels, navel-like blossom-ends, zippering, and open locules was

Table 3-4. Pearson correlation coefficients (r) between blossom-end scar index (BSI) and fruit size 14 days after anthesis, number of seeds per fruit, and number of locules per fruit for each cultivar.

	Horizon		Solar Set		Valerie	
	r	*P* value	r	*P* value	r	*P* value
Early fruit size	-0.56	0.116	-0.76	0.018	-0.45	0.226
Seed number	0.92	0.009	0.90	0.014	0.51	0.298
Locule number	0.66	0.157	0.24	0.648	0.94	0.005

Table 3-5. Analysis of variance for transformed data ($\log_{10}(x+1)$) for the percentages of fruits with channels, navel-like blossom-ends, zippering, and open locules.

Source	Channels			Navels		
	df	MS	*P* value	df	MS	*P* value
Replication	3	0.0229	0.148	3	0.0440	0.037
Cultivar	2	3.9308	<0.001	2	0.7310	<0.001
Growth regulator treatment	2	0.0356	0.067	2	0.0174	0.290
Cultivar x treatment	4	0.0017	0.965	4	0.0243	0.157
Error	24	0.0117		24	0.0133	
Total	35			35		

Table 3-5. Continued

Source	Zippering			Open locules		
	df	MS	*P* value	df	MS	*P* value
Replication	3	0.0048	0.815	3	0.0018	0.841
Cultivar	2	0.4665	<0.001	2	0.0258	0.034
Growth regulator treatment	2	0.0145	0.404	2	0.0351	0.012
Cultivar x treatment	4	0.0041	0.899	4	0.0054	0.530
Error	24	0.0154		24	0.0066	
Total	35			35		

calculated. ANOVA according to model II indicated that interaction effects were insignificant ($\alpha = 0.10$; Table 3-5). The cultivars in this study differed significantly in susceptibility to each malformation ($\alpha = 0.05$; Table 3-5). 'Horizon' had the highest and 'Valerie' the lowest occurrence of channels and navels (Table 3-6), leading to the impression that occurrence of these blossom-end disorders might be related to susceptibility for RBS. Fortney (1958) reported a significant correlation between the number of scar openings and scar size (r = 0.5 to 0.7). The occurrence of zippers and open locules was highest in 'Solar Set' and lowest in 'Valerie' (Table 3-6). The differences between growth regulator treatments were generally insignificant; however, the 4-CPA treatment markedly increased the occurrence of open locules in comparison to the GA_3 and control treatments (Table 3-6). Zalik et al. (1951) observed a very similar 'strawberry effect' after direct application of 4-CPA on the ovary shortly before anthesis.

Table 3-6. Average percentage of fruits with channels, navel-like blossom-ends, zippering, and open locules per cultivar and growth regulator treatment.

	Total fruits harvested (no.)	Fruits with malformation			
		Channels (%)	Navels (%)	Zippers (%)	Open locules (%)
Cultivar					
Horizon	3937	34.98 a[z]	2.48 a	0.59 b	0.18 ab
Solar Set	4368	25.40 b	1.39 b	1.62 a	0.34 a
Valerie	3285	2.35 c	0.08 c	0.02 c	0.06 b
Growth regulator treatment					
Control	4298	21.74 a	1.44 a	0.80 a	0.14 a
GA_3	3456	22.95 a	1.43 a	0.55 a	0.06 a
4-CPA	3836	18.05 a	1.08 a	0.89 a	0.39 b

[z] Averages indicated with the same letter are not significantly different in Duncan's multiple range test with $\alpha = 0.05$.

Discussion

Much attention has been directed towards the usefulness of GA_3 and 4-CPA as fruit set promoting substances under unfavorable environmental conditions. Treatment of inflorescences with GA_3 promoted parthenocarpic fruit set in emasculated flowers (Sastry and Muir, 1963; Wittwer et al., 1957) and under unfavorable conditions (Abdalla and Verkerk, 1970; Gustafson, 1960), but fruits developed slowly and mature fruits were small (Abdalla and Verkerk, 1970; Bangerth and Sjut, 1978). In the present study under favorable environmental conditions, the GA_3 treatment did not affect the average number of fruits set per plant; however, pairwise comparison between the GA_3 and the control treatment indicated that application of GA_3 did result in significantly slower early fruit growth. No parthenocarpic fruit set ($\leq$ 8 seeds; Varga and Bruinsma, 1976) occurred after GA_3 treatment, which agrees with the results of Mapelli et al. (1979), who reported the absence of parthenocarpic fruit set after GA_3 treatment of unemasculated flowers. Treatment with 4-CPA did not affect early fruit growth (Table 3-3), but mature fruits from 4-CPA treated plots were larger than from control plots, probably as the result of a prolonged cell division period (Bünger-Kibler and Bangerth, 1983). Increased fruit size as a result of 4-CPA treatment during flowering has frequently been reported (e.g., Mullison and Mullison, 1948; Wittwer et al., 1948). Four percent of the 4-CPA treated fruits examined were parthenocarpic, agreeing with the results of Verkerk (1957), who reported increased seedlessness after 4-CPA treatment under conditions favorable for fruit set.

Dramatic differences existed among cultivars in sensitivity to RBS induction (Figure 3-1) and improvement through breeding seems a promising approach. Previous studies indicated that the inheritance of blossom-end scar size is additive and that genotype x environment interactions are of little importance (Barten et al., 1991b). It is also apparent from Figure 3-1 that application of $1.3 \cdot 10^{-4}$ M 4-CPA cannot counteract RBS induction by low temperatures under field conditions. However, 4-CPA application might result in a slight, insignificant reduction in occurrence of channels and navel-like blossom-ends (Table 3-6). The effect of $2.9 \cdot 10^{-5}$ M GA_3 on RBS induction in flowering plants is strongly influenced by temperature conditions during early flower differentiation (Figure 3-1). It seems advisable to work with controlled temperature regimes for further research of the physiological backgrounds of RBS. Growth regulator treatments affected flowers that were at anthesis from 5-10 April for 'Horizon', and from 10-15 April for 'Solar Set' (Figure 3-1). Thus, 'Horizon' flowers were sensitive to the growth regulator treatments 4 days before to 1 day after anthesis, whereas 'Solar Set' flowers were sensitive 9-4 days before anthesis. This difference in sensitive period between 'Horizon' and 'Solar Set' may be due to interaction of the growth regulator treatments with the low temperature induction of RBS in flowers that were at anthesis from 10-16 April (Figure 3-1). No information was obtained on sensitivity of flowers that were more than 1 day after anthesis or more than 14 days before anthesis at the time of growth regulator treatment.

Wien and Zhang (1991) reported recently that $5.0 \cdot 10^{-6}$ M GA_3 applied at transplanting caused an increase in average RBS in fruit of the first six clusters of the susceptible cultivar Revolution. No information was available on the severity of RBS in individual clusters. Apparently, application of GA_3 at transplanting induces RBS in fruits that are not initiated yet at the time of treatment. GA_3 applied at transplanting is known to have a long term effect on plant growth and morphology by promoting vegetative growth and decreasing flower numbers (Abdul and Harris, 1978; Abdul et al., 1978; Bukovac et al., 1957), and reducing fruit growth (Brown et al., 1968). Thus, a single application of GA_3 at transplanting might have a long term effect on RBS induction. In the present experiment involving young generative plants, only flowers at a specific developmental stage seem to be affected by the growth regulator treatments. GA_3 applied to young generative plants may induce RBS in flowers that are 26-19 days before anthesis at the time of treatment, similarly to the short term low temperature effect reported by Barten et al. (1991a); however, this hypothesis could not be tested in the present experiment due to low fruit numbers.

The role of seed numbers in RBS induction was investigated. The presence of IAA in young ovaries has been associated with developing seeds (Varga and Bruinsma, 1976). Increased seed numbers caused faster initial fruit growth (Varga and Bruinsma, 1976) and heavier mature fruits (Dempsey and Boynton, 1965; Verkerk, 1957). If RBS were the result of reduced auxin activity in developing ovaries due to poor seed set, a negative correlation between BSI and seed numbers would be expected. However, the correlations between average BSI and average seed numbers were significantly positive for 'Horizon' and 'Solar Set' (Table 3-4). Treatment with 4-CPA may have substituted auxin activity related to young developing seeds and thus biased the correlations. The Pearson correlation coefficients (r) between BSI and seed number for individual fruits in the control treatment were insignificant; r = 0.00, r = 0.15, and r = 0.03 for 'Horizon', 'Solar Set', and 'Valerie', respectively. In a related study, significant differences in seed numbers were obtained by pollination with diluted pollen; however, BSI was not significantly affected by the treatments (Appendix B). This agrees with literature suggesting that RBS induction occurs before anthesis (Barten et al., 1991a; Knavel and Mohr, 1969), and not during or after anthesis (Rylski, 1979; Sikes and Coffey, 1976). Occasionally, poor pollination in one or more locules may affect fruit shape and aggravate problems with blossom-end closure during fruit development (Sikes and Coffey, 1976; Appendix C).

Increased locule numbers have been suggested as the cause of severe low temperature induced fruit malformations (Asahira et al., 1982; Hosoki et al., 1985) and RBS (Wien and Zhang, 1991). Sawhney (1984) reported that GA_3 increased the number of locules per fruit when applied during flower initiation. In the present experiment, treatment with neither GA_3 nor 4-CPA affected the number of locules in ovaries that were 4 or 6 days before anthesis at the time of application. The insignificant correlations between average BSI and average locule number for 'Horizon' and 'Solar Set' (Table 3-4) agree with results of Barten et al. (1991a), who reported that in their material RBS was not caused by increased locule numbers. The correlation between average BSI and average locule numbers was significantly positive for 'Valerie' (Table 3-4); however, this cultivar had very little variation in BSI and the importance of the correlation was limited. The average number of locules per cultivar (Table 3-3) was inversely related to sensitivity to RBS induction (Figure 3-1). In a related study, the correlation between the average number of locules per fruit and average BSI across 17 cultigens was insignificant (Appendix A).

Russel and Morris (1983) studied assimilate distribution in young generative tomato plants by application of [^{14}C]sucrose to source leaves. They reported that shoot apices and roots were in competition with developing inflorescences for assimilates. This may explain why restriction of root growth (Cooper and Hurd, 1968) or removal of young leaves (Kinet, 1977; Russel and Morris, 1982) promoted development of the first inflorescences. Growth retardants, such as 2-chloroethyltrimethylammonium chloride, reduced shoot growth, but promoted growth and development of inflorescences (Abdalla and Verkerk, 1970; Nourai and Harris, 1983). These workers suggested that the growth retardants influenced the competition for assimilates in favor of developing inflorescences. In the present study, GA_3 applied to young flowering plants inhibited early fruit growth and promoted shoot growth, suggesting that GA_3 might influence the partitioning of assimilates in favor of shoot growth. This may be related to the reduction in cell division in developing ovaries (Bünger-Kibler and Bangerth, 1983) and increased RBS (Figure 3-1B) after GA_3 application. The negative correlation between average BSI and average early fruit size (Table 3-4) fits the hypothesis that RBS is caused by reduced cell division activity at the stylar base, preventing complete closure of the carpel walls. The RBS induction by short term low temperatures during early flower development reported by Barten et al. (1991a) may have been caused by a reduction in cell division during a critical stage of development. Short term low temperatures are known to promote assimilate partitioning to the stem and roots at the cost of developing flowers (Shishido and Hori, 1979; Yoshioka and Takahashi, 1981).

CHAPTER 4
DIALLEL ANALYSIS OVER TWO LOCATIONS FOR BLOSSOM-END SCAR SIZE IN TOMATO

Introduction

Rough blossom-end scarring (RBS) in tomato (*Lycopersicon esculentum* Mill.) is characterized by the development of corky scar tissue at the stylar base, usually containing channels that lead to internal locular tissue. RBS reduces aesthetic value and shelf-life of the fruit due to susceptibility to bruising, leaking of fruit juices, and post-harvest infections. The disorder always occurs to some extent wherever large fruited tomatoes are grown, often leading to substantial economic losses for the fresh market tomato industry (Barksdale et al., 1972). External stress factors, such as low temperatures (Knavel and Mohr, 1969; Rylski, 1979), pruning (Sikes and Coffey, 1976; Wien and Minotti, 1988), and possibly winds (Young and McArthur, 1947) have been associated with RBS.

Not much is known about the genetic regulation of blossom-end scar size. Fortney (1958) reported that the number of stylar scar openings was inherited as a quantitative character with additive gene action and heritability estimates of 0.25 and 0.55 for two different crosses. The correlation between the number of scar openings and scar size was significant (r = 0.5 to 0.7). However, Fortney (1958) investigated only two crosses and the genetic regulation of blossom-end scar size was not studied directly. Elkind et al. (1990b) reported significant genetic variation in blossom-end scar size for 27 homogeneous genotypes consisting of 18 pure lines and 9 F1's. Heritability values were calculated using estimated variance components of genetic and environmental effects. Heritability estimates for the 18 pure lines (= narrow sense heritability) were 0.88 - 0.93 based on single plots, and 0.82 - 0.86 based on single plants (Elkind et al., 1990b). These high levels of heritability were probably due to large genetic variation. A stability analysis involving four cultigens grown in six fields in Israel indicated that the significant genotype x environment interaction for blossom-end scar size was mostly due to heterogeneity of the stability slopes and that the ranking of cultivars was consistent across all environments (Elkind et al., 1990a).

Although RBS was observed under low as well as high temperatures, it was not clear whether the genetic regulation was the same under both conditions. To investigate this, half diallel crosses were grown under cool winter conditions in Israel and warm fall conditions in Florida. The genetic regulation of blossom-end scar size in each climate was investigated as well as the stability of genetic parameters over the two locations. Diallel crosses were used in this experiment because in both countries much breeding work is concentrated on combining useful characteristics in F1 hybrid cultivars.

Materials and Methods

The half diallel cross in Bradenton, Florida, consisted of 11 parents representing a wide array of susceptibility to RBS (Table 4-1) and 55 F1's. Parental and F1 generations were planted in the field on 24 August 1987 and raised under high temperatures common in the fall (minimum 12C-21C; maximum 24C-30C). The 66 genotypes were grown in randomized 5-plant plots in three replications. From most plots, 40 mature fruits were harvested; however, some plots were damaged by herbicides and yielded less fruits, but at least 15. No fruits could be harvested in seven plots.

In the half diallel cross in Israel one parent, Fla 8181, was omitted. Thus, the experiment consisted of 10 parental and 45 F1 generations, grown in randomized 3-plant plots in three replications.

Table 4-1. Parents used in diallel.

Parent	Blossom-end scar Subjective rating
NC 8276[z]	Smooth
NC 140[z]	Smooth
Fla 8041[y]	Intermediate
Fla 8181[y]	Intermediate
Fla MH-1[y]	Intermediate
Walter[y]	Intermediate
Fla 7131[y]	Intermediate
Suncoast[y]	Rough
Fla 7181[y]	Rough
Horizon[y]	Rough
Fla 7132[y]	Rough

[z] From breeding program at North Carolina State University.

[y] From breeding program at University of Florida.

The plants were planted at Hazeva in the Arava on 19 October 1987 and grown under low temperatures in the winter (minimum 5C-12C; maximum 13C-24C). To promote fruit set, the plants were treated with 0.3% Oraset (20% N-Meta-toly-Phtalamic Acid). From each plot 25 mature fruits were harvested.

For individual fruits, the blossom-end scar size was measured, relative to fruit size as follows:

$$BSI = \frac{\sqrt{a \times b}}{\sqrt{c \times d}} \times 100$$

with BSI = blossom-end scar index,

a = largest diameter of blossom-end scar,

b = smallest diameter of blossom-end scar,

c = largest diameter of equatorial fruit cross section,

d = smallest diameter of equatorial fruit cross section.

The BSI values were averaged per plot before diallel analysis. Least squares estimates for the missing plot values in Bradenton were obtained using PROC GLM in SAS (Statistical Analysis System) and included in the analysis.

The diallel crosses were analyzed per location according to Griffing (1956) and Hayman (1954). General (GCA) and specific combining ability (SCA) estimates were calculated according to Griffing's experimental method 2 (parents and one set of F1's included). For testing procedures, compliance with assumptions for analysis of variance (ANOVA) was necessary (Griffing, 1956). Information on significance of additive and dominance gene effects, epistatic effects, general direction and nature of dominance, and heritabilities was obtained with the Hayman diallel analysis based on covariances (Wr) and variances (Vr) of parental arrays. Assumptions for Hayman analysis were: diploid segregation, homozygous parents, independent gene distribution, and absence of nonallelic interaction, reciprocal differences, and multiple alleles. The first two assumptions were met for the genetic material in this experiment. Furthermore, extensive breeding experience with RBS did not indicate the presence of reciprocal differences. The other assumptions were tested by inspecting the Vr, Wr regression and by Hayman's (1954) t^2 test. Broad sense and narrow sense heritabilities were calculated according to Mather and Jinks (1971). Stability of genetic parameters over locations was analyzed according to Allard (Allard, 1956; Crumpacker and Allard, 1962).

Results

Griffing Analysis

Preliminary ANOVA indicated that BSI values were significantly different between genotypes (Table 4-2). Plots of residuals versus fitted values did not indicate violation of ANOVA assumptions in either location and untransformed data were averaged over replications per location (Tables 4-3 and 4-4). NC 8276 and NC 140 had desirable, low BSI values in both locations. 'Suncoast', Fla 7181, 'Horizon', and Fla 7132 had high values for BSI in both locations; however, for 'Suncoast' the average BSI was much higher in Hazeva than in Bradenton (Table 4-3). NC 8276 x 'Walter', NC 140 x NC 8276, NC 140 x Fla 8041, NC 140 x

Table 4-2. Analysis of variance for blossom-end scar index in a 11 x 11 diallel cross at Bradenton and a 10 x 10 diallel cross at Hazeva.

	Bradenton			Hazeva		
Source	df	MS	*P* value	df	MS	*P* value
Replication	2	0.88	0.503	2	0.37	0.770
Genotype	65	11.95	<0.001	54	19.57	<0.001
Error	123	1.27		108	1.39	
Total	190[z]			164		

[z] The total degrees of freedom were reduced by 7, because 7 values were least square estimates.

Table 4-3. Mean performance of parents for blossom-end scar index (BSI).

Bradenton			Hazeva		
Parent	Avg BSI[z]	DMRT[y]	Parent	Avg BSI[z]	DMRT[y]
NC 8276	4.37	a	NC 140	3.61	a
NC 140	5.57	ab	NC 8276	3.64	a
Fla 8041	6.14	ab	Fla MH-1	5.08	ab
Fla 8181	6.66	b	Walter	5.25	ab
Fla MH-1	6.87	b	Fla 7131	6.64	bc
Walter	7.24	bc	Fla 8041	6.67	bc
Fla 7131	8.80	cd	Fla 7181	8.06	c
Suncoast	9.94	de	Horizon	10.88	d
Fla 7181	11.09	ef	Fla 7132	11.86	d
Horizon	11.15	ef	Suncoast	16.24	e
Fla 7132	12.91	f			

[z] Standard error for average BSI is 0.65 in Bradenton and 0.68 in Hazeva.

[y] Duncan's multiple range test, means followed by the same letter are not significantly different at $\alpha = 0.05$.

Table 4-4. Mean performance of hybrids for blossom-end scar index (BSI). Above diagonal for Bradenton, below diagonal for Hazeva.

Parent	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
1) NC 8276	-	4.82	4.94	4.56	3.93	5.90	6.77	6.97	6.66	6.43	7.26
2) NC 140	4.27	-	6.01	5.38	5.49	5.91	7.05	5.92	6.92	6.92	7.78
3) Fla 8041	4.54	4.32	-	4.70	4.66	7.40	7.53	6.71	9.78	8.21	9.71
4) Fla 8181	-	-	-	-	6.59	6.63	7.51	7.76	8.17	8.90	10.06
5) Fla MH-1	6.20	4.89	5.58	-	-	8.06	6.83	7.55	8.44	8.33	7.65
6) Walter	5.15	3.76	4.94	-	6.38	-	7.49	7.26	6.76	6.60	7.16
7) Fla 7131	7.75	3.87	7.49	-	7.42	5.19	-	7.00	11.02	7.33	9.54
8) Suncoast	5.60	5.27	7.82	-	8.78	8.20	9.82	-	11.06	9.22	10.95
9) Fla 7181	6.75	4.80	9.63	-	8.33	6.35	8.48	11.22	-	12.13	10.61
10) Horizon	6.21	5.41	8.04	-	8.01	6.79	8.78	11.56	9.27	-	9.88
11) Fla 7132	7.92	5.73	9.03	-	9.95	7.55	8.97	12.17	9.01	9.65	-

Standard error for average BSI is 0.65 in Bradenton and 0.68 in Hazeva.

'Walter', and NC 140 x 'Florida MH-1' ranked in the top ten performing hybrids in both locations (low BSI in Table 4-4). NC 140 apparently produced hybrids with low BSI. In Hazeva, the performance of NC 140 and NC 8276 was better than any hybrid combination. The average BSI of 7.60 in Bradenton was significantly higher than the average BSI of 7.36 in Hazeva ($\alpha = 0.05$).

ANOVA according to Griffing (1956) indicated that in both locations GCA and SCA effects were highly significant (Table 4-5). NC 8276 and NC 140 had desirable, negative GCA values in both locations (Table 4-6). The GCA values were significantly positive for 'Suncoast', 'Horizon', Fla 7181, and Fla 7132 in both locations (Table 4-6). Except for 'Suncoast', the ranking of parents according to GCA was similar over locations (Table 4-6). According to Baker (1978), the relative importance of parental GCA in predicting progeny performance can be evaluated using the variance ratio $2S^2_{GCA}/(2S^2_{GCA} + S^2_{SCA})$, where the

Table 4-5. Combining ability analysis for blossom-end scar index in a 11 x 11 diallel cross at Bradenton and a 10 x 10 diallel cross at Hazeva.

	Bradenton			Hazeva		
Source	df	MS	*P* value	df	MS	*P* value
GCA	10	20.59	<0.001	9	32.56	<0.001
SCA	55	0.96	<0.001	45	1.31	<0.001
Error	123	0.42		108	0.46	
Total	190[z]			164		
S^2_{GCA}		1.55			2.68	
S^2_{SCA}		0.54			0.85	
$2S^2_{GCA}/(2S^2_{GCA} + S^2_{SCA})$		0.85			0.86	

[z] The total degrees of freedom were reduced by 7, because 7 values were least square estimates.

Table 4-6. Estimates of general combining ability ($\hat{g}_i$) of parents for blossom-end scar index.

Bradenton			Hazeva		
Parent	$\hat{g}_i$[z]	DMRT[y]	Parent	$\hat{g}_i$[z]	DMRT[y]
NC 8276	-1.86	a	NC 140	-2.62	a
NC 140	-1.38	b	NC 8276	-1.61	b
Fla MH-1	-0.77	c	Walter	-1.35	b
Fla 8041	-0.71	c	Fla 8041	-0.52	c
Fla 8181	-0.59	c	Fla MH-1	-0.44	c
Walter	-0.58	c	Fla 7131	0.01	c
Fla 7131	0.35	d	Fla 7181	0.75	d
Suncoast	0.70	de	Horizon	1.21	d
Horizon	1.16	e	Fla 7132	1.90	e
Fla 7181	1.73	f	Suncoast	2.66	f
Fla 7132	1.94	f			

[z] Standard error for $\hat{g}_i$ is 0.17 in Bradenton and 0.19 in Hazeva.

[y] Duncan's multiple range test, means followed by the same letter are not significantly different at $\alpha = 0.05$.

variance components S^2_{GCA} and S^2_{SCA} are calculated from the expected components of mean squares assuming a fixed effects model (Griffing, 1956). This ratio was close to unity in both locations (Table 4-5), indicating that hybrid performance could be predicted to a great extent by GCA of the parents. Both S^2_{GCA} and S^2_{SCA} were higher in Hazeva than in Bradenton (Table 4-5), suggesting more additive and dominance variation in Hazeva than in Bradenton. An important question for plant breeders is whether the mean performance of parents is indicative of their GCA. Following Mohanty and Khush (1985), the Pearson correlation coefficient between parental mean and GCA was 0.99 in Bradenton and 0.95 in Hazeva. High predictability of hybrid performance based on GCA of the parents as well as a high correlation of parent performance with GCA suggest important additive effects.

The SCA of the cross between the two top performing parents (NC 8276 and NC 140) was positive in both locations (Table 4-7). The SCA differed significantly between the two locations in nine crosses (α = 0.05). These crosses involved NC 8276, Fla 7131, and 'Walter' as at least one of the parents. The variation in SCA estimates for the array of crosses involving one common parent was compared between parents (Table 4-8). The two parent lines with desirable GCA values (NC 8276 and NC 140) had a low variation in SCA only in Bradenton. This means that in the diallel in Bradenton, NC 8276 and NC 140 transmitted smoothness (low BSI) uniformly to their F1's. The variance in SCA for NC 8276 was high in Hazeva, indicating that some hybrid combinations with NC 8276 as parent performed considerably better than predicted on the basis of GCA and others considerably worse. In Hazeva, NC 140 transmitted smoothness more uniformly than NC 8276. For the parents with unfavorable GCA, the variance in SCA varied widely from 0.33 ('Horizon' in Hazeva) to 3.11 ('Suncoast' in Hazeva).

Hayman Analysis

From the data in Tables 4-3 and 4-4, Vr (the variance of the array with common parent r) and Wr (the covariance between the parental array and the offspring with recurring parent r) were calculated and used to graphically analyze dominance relationships in the diallel cross (Figure 4-1). Hayman (1954) showed that $Wr - Vr = 1/4(D - H_1)$, where D and H_1 are the components of variation due to additive and dominance effects, respectively. Thus, if the assumptions for Hayman analysis are valid,

Table 4-7. Estimates of specific combining ability ($\hat{s}_{ij}$) for blossom-end scar index. Above diagonal for Bradenton, below diagonal for Hazeva.

Parent	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
1) NC 8276	-	0.45	-0.08	-0.59	-1.04	0.75	0.69	0.53	-0.81	-0.47	-0.42
2) NC 140	1.14	-	0.50	-0.26	0.03	0.26	0.48	-1.00	-1.04	-0.46	-0.39
3) Fla 8041	-0.69	0.10	-	-1.61	-1.46	1.09	0.30	-0.88	1.16	0.17	0.88
4) Fla 8181	-	-	-	-	0.34	0.20	0.15	0.05	-0.58	0.73	1.11
5) Fla MH-1	0.89	0.59	-0.82	-	-	1.80	-0.35	0.02	-0.13	0.34	-1.13
6) Walter	0.74	0.36	-0.56	-	0.80	-	0.12	-0.46	-1.99	-1.58	-1.80
7) Fla 7131	1.99	-0.88	0.64	-	0.49	-0.83	-	-1.64	1.35	-1.77	-0.35
8) Suncoast	-2.82	-2.14	-1.69	-	-0.81	-0.48	-0.21	-	1.03	-0.23	0.71
9) Fla 7181	0.25	-0.69	2.04	-	0.66	-0.42	0.36	0.45	-	1.64	-0.66
10) Horizon	-0.75	-0.54	-0.01	-	-0.12	-0.44	0.20	0.33	-0.05	-	-0.82
11) Fla 7132	0.27	-0.91	0.29	-	1.13	-0.36	-0.29	0.22	1.00	0.82	-

Standard error for $\hat{s}_{ij}$ is 0.60 in Bradenton and 0.63 in Hazeva,
Standard error for ($\hat{s}_{ij}$ - $\hat{s}_{ik}$) is 0.88 in Bradenton and 0.92 in Hazeva,
Standard error for ($\hat{s}_{ij}$ - $\hat{s}_{kl}$) is 0.85 in Bradenton and 0.88 in Hazeva,
where i,j,k and l indicate the parents of a hybrid.

Table 4-8. Variance in specific combining ability estimates for crosses with one common parent.

Parent	Bradenton	Hazeva
NC 8276	0.42	1.77
NC 140	0.37	1.19
Fla 8041	0.92	1.01
Fla 8181	0.53	-
Fla MH-1	0.87	0.76
Walter	1.56	0.37
Fla 7131	0.90	0.76
Suncoast	0.74	3.11
Fla 7181	1.34	0.80
Horizon	1.15	0.33
Fla 7132	1.04	0.52

the points (Vr, Wr) lie on a straight line with unit slope and intercept 1/4(D - H_1). Statistical limitations imply that all data points in the Vr, Wr graph must lie inside the parabola $Wr^2 \leq V_{OLO} \cdot Vr$, where V_{OLO} is the variance of the parents. Deviation of the regression coefficient (b) from unit slope was insignificant and Hayman's (1954) t^2 test indicated constancy of Wr - Vr in both locations (Figure 4-1 and Table 4-9). This means that Hayman's assumptions for diallel analysis were met and that epistatic effects were not important in genetic regulation of BSI. The positive intercepts of the regression lines with the Y-axis in Figure 4-1 indicated that dominance was on the average incomplete.

Prevailing dominant gene effects in parent r result in relatively low variance within its progeny (Vr) and relatively low covariance of its progeny with the nonrecurrent, more recessive parents in the analysis (Wr). Thus, parents with primarily dominant gene effects correspond with points at the lower end of the regression line and

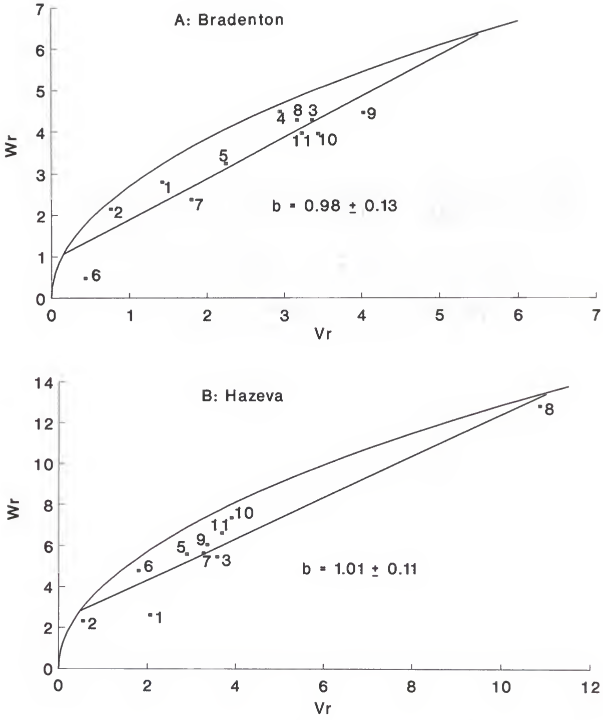

Figure 4-1. Vr, Wr graph of Hayman analysis for blossom-end scar index in Bradenton (A) and Hazeva (B). 1 = NC 8276; 2 = NC 140; 3 = Fla 8041; 4 = Fla 8181; 5 = Florida MH-1; 6 = Walter; 7 = Fla 7131; 8 = Suncoast; 9 = Fla 7181; 10 = Horizon; 11 = Fla 7132. The limiting parabola is $Wr^2 = 7.35 \cdot Vr$ in Bradenton and $Wr^2 = 16.52 \cdot Vr$ in Hazeva.

parents with primarily recessive gene effects correspond with points at the upper end. For 'Suncoast', recessive gene effects were more important in Hazeva than in Bradenton, whereas in the other parents the relative proportion of dominance was greater in Hazeva than in Bradenton (Figure 4-1). Except for 'Suncoast', the values for Vr and Wr were similar over locations.

Genetic parameters and their standard errors were estimated using the procedures of Hayman (1954; Table 4-9). Significance of the F parameter suggests unequal allelic distribution among the parents with $F > 0$ when dominant alleles are in excess and $F < 0$ when recessive alleles are in excess. $H_2/4H_1$ estimates the mean value of $u_i \times v_i$ with u_i = the proportion of positive and v_i = the proportion of negative alleles at locus *i* (causing higher and lower BSI, respectively), and $u_i + v_i = 1$. When positive and negative alleles are equally distributed over the parents for each locus, $u_i = v_i = 1/2$ for all *i*, $H_2/4H_1 = \overline{u_i \times v_i} = 1/4$, and $H_1 = H_2$ (Christie et al., 1988). In Bradenton, the distribution of alleles over the parents was equal for all loci as indicated by F, H_1, and H_2 parameters (Table 4-9). In Hazeva, however, H_1 was significantly greater than H_2, and $H_2/4H_1 < 0.25$, indicating unequal allelic distribution for at least one locus. Furthermore, a significantly positive F value indicated an excess of dominant alleles in the parents in Hazeva (Table 4-9). The change in the distribution of alleles between locations may be due to absence of Fla 8181 from the diallel in Hazeva; however, elimination of this parent from the diallel in Bradenton did not result in unequal allele distribution (Table 4-9). The disturbance of equal allelic distribution in Hazeva may be explained

Table 4-9. Estimations and standard error of genetic parameters for Hayman analysis of blossom-end scar index.

Parameter^{z}	Bradenton			Hazeva	
	All parents included	Fla 8181 excluded	Fla 8181 & 'Suncoast' excluded	All parents included	'Suncoast' excluded
n^{y}	11	10	9	10	9
t^{2}	$0.25^{NS,x,w}$	0.14^{NS}	0.04^{NS}	0.24^{NS}	0.00^{NS}
b^{v}	0.98 ± 0.13	0.98 ± 0.13	0.95 ± 0.15	1.01 ± 0.11	0.94 ± 0.12
E	0.42 ± 0.10^{*u}	0.43 ± 0.11*	0.47 ± 0.13*	0.46 ± 0.19*	0.38 ± 0.07*
D	6.93 ± 0.34*	7.43 ± 0.35*	8.05 ± 0.42*	16.05 ± 0.62*	8.29 ± 0.23*
F	0.77 ± 0.78^{NS}	1.28 ± 0.81^{NS}	1.86 ± 0.99^{NS}	8.52 ± 1.43*	2.28 ± 0.54*
H_1	2.69 ± 0.70*	3.10 ± 0.75*	3.22 ± 0.93*	5.83 ± 1.32*	3.04 ± 0.51*
H_2	2.72 ± 0.59*	2.93 ± 0.64*	2.91 ± 0.80*	3.23 ± 1.12*	1.86 ± 0.44*
$D-H_1$	4.24 ± 0.56*	4.33 ± 0.60*	4.83 ± 0.76*	10.22 ± 1.06*	5.25 ± 0.41*
$H_2/4H_1$	0.25	0.24	0.23	0.14	0.15
$(H_1/D)^{1/2}$	0.62	0.65	0.63	0.60	0.61
h	-1.42	-1.51	-1.41	-0.95	-0.02
$r_{Yr,(Wr+Vr)}$	0.51	0.65	0.61	0.92	0.54
N.S.^{t}	0.74	0.73	0.73	0.80	0.81
B.S.^{t}	0.90	0.90	0.89	0.93	0.91

[z] Parameters as described in Hayman (1954).

[y] Number of parents in diallel.

[x] NS,*: Nonsignificant or significant at $\alpha = 0.05$.

[w] Significance of the t^2 statistic indicates failure of Hayman's (1954) hypotheses.

[v] Regression coefficient in Vr, Wr graph; significant deviation from unit slope indicates failure of Hayman's (1954) hypotheses.

[u] Significance of Hayman parameters was tested with Student's t-test with n-1 degrees of freedom. Significance of the difference between parameters in different analyses was tested by constructing 95% confidence intervals using $t_{0.05}$ with n-1 degrees of freedom.

[t] N.S.: Narrow sense heritability; B.S. : Broad sense heritability.

by the fact that the analysis detected gene action in Hazeva, which remained undetected in Bradenton. In other words, the climate in Hazeva may have activated recessive gene action in 'Suncoast' that was inactive in Bradenton (Figure 4-1). Following this hypothesis, dominant gene action activated in the other parents in Hazeva (Figure 4-1) may explain the significantly positive F parameter in Hazeva (Table 4-9). After exclusion of 'Suncoast' from the diallel in each location, the allele distribution in Hazeva was still unbalanced; however, none of the parameters F, H_1, and H_2 was significantly different between Bradenton and Hazeva (Table 4-9).

Additive and dominance effects were both significant, but additive effects were the main cause of genetic variation in this experiment (Table 4-9). Additive effects were significantly larger in Hazeva than in Bradenton when all parents were included. However, when 'Suncoast' was excluded from the diallel in each location, the difference in additive effects between Bradenton and Hazeva was insignificant (Table 4-9). This is probably related to additional gene action in Hazeva, which is only detectable when 'Suncoast' is included in the analysis. Estimations of $D - H_1$ indicated partial dominance and the mean degree of dominance over all loci, estimated with $(H_1/D)^{1/2}$, varied from 0.60 to 0.65 (Table 4-9). The dominance effects summed over all loci, indicated as h, can be calculated as $2(M_{L1} - M_{L0})$, where M_{L0} and M_{L1} are the average BSI in the parents and progeny, respectively (Hayman, 1954). Values for h were negative in both locations, indicating that the dominance effects in the direction of lower BSI were most important. Hayman (1954) showed that (Wr + Vr) is closely correlated with the number of recessive homozygotes in the parents, whereas the parental value (Yr) is indicative of the number of positive homozygotes in the parents. The correlation between Yr and (Wr + Vr) then indicates whether mainly recessive ($r_{Yr.(Wr+Vr)} < 0$) or dominant ($r_{Yr.(Wr+Vr)} > 0$) alleles are negative. In Bradenton, this correlation was positive but small, indicating that individual dominant alleles may be positive as well as negative, but that the general tendency was towards negative. In Hazeva, this correlation was strongly inflated by prominent recessive effects in 'Suncoast', combined with high values for BSI. The high heritabilities in Table 4-9 were caused by averaging data over three replications before analysis. However, single plot heritability estimates may be more appropriate since selections are generally made on the basis of single plots. Single plot estimates were 0.61 and 0.70 for narrow sense, and 0.75 and 0.81 for broad sense heritability in Bradenton and Hazeva, respectively.

Allard Analysis

Stability of additive effects over the two locations in this experiment was investigated by means of ANOVA of the parents common to both locations. Because of the dramatic difference in 'Suncoast' over locations reported previously, analysis was performed with and without 'Suncoast'. When 'Suncoast' was included in the analysis, location x genotype interaction was significant (Table 4-10), suggesting that additive effects interacted with locations. However, after elimination of 'Suncoast', the location x genotype interaction was insignificant,

Table 4-10. Analysis of variance over locations for blossom-end scar index of the parents common to Bradenton and Hazeva.

	'Suncoast' included (10 parents)			'Suncoast' excluded (9 parents)		
Source	df	MS	*P* value	df	MS	*P* value
Location	1	5.7	0.031	1	25.9	<0.001
Genotype	9	62.7	<0.001	8	49.8	<0.001
Location x genotype	9	10.5	<0.001	8	1.8	0.143
Error	40	1.1		36	1.1	
Total	59			53		

indicating constancy of additive effects over locations (Table 4-10). Apparently, 'Suncoast' was the only cause of significant interaction of parental genotypes with the locations. Significance of the genotype component of variation indicated that BSI values were significantly different between the parents (Table 4-10). After exclusion of 'Suncoast', BSI values averaged over the parents were significantly different between Hazeva and Bradenton, as indicated by significant location effect in Table 4-10.

Vr and Wr values for the subdiallel common to both locations were used to investigate the interaction of dominance effects with environment. Allard (1956) argues that dividing Vr and Wr by the variance of the parents occurring in the same replication optimizes the efficiency of the test. Significance of the location x array interaction in ANOVA of adjusted Vr and Wr values suggested instability of dominance effects over locations (Table 4-11). However, insignificant location x array interation in ANOVA without 'Suncoast' indicated that dominance effects were constant over locations (Table

Table 4-11. Analysis of variance over locations of adjusted Vr and Wr for parents common to Bradenton and Hazeva.

	'Suncoast' included (10 x 10 diallel)			'Suncoast' excluded (9 x 9 diallel)		
Source	df	MS ($x10^{-2}$)	*P* value	df	MS ($x10^{-2}$)	*P* value
Location	1	23.6	<0.001	1	1.8	0.431
Dominance	1	8.1	0.038	1	3.1	0.298
Location x dominance	1	2.0	0.301	1	0.1	0.854
Array	9	27.4	<0.001	8	21.7	<0.001
Location x array	9	6.9	<0.001	8	4.0	0.211
Array x dominance	9	0.3	0.996	8	0.7	0.982
Loc. x array x dom.	9	1.1	0.808	8	0.8	0.970
Error	80	1.8		72	2.8	
Total	119			107		

4-11). Apparently, 'Suncoast' was the major cause for interaction of dominance effects with the two locations. The arrays component of variation suggested significant difference in dominance among the parents of the diallel. Insignificant array x dominance, location x dominance, and location x array x dominance sources of variation indicated absence of epistatic effects, constancy of mean dominance, and constancy of nonallelic interactions over locations, respectively (Table 4-11).

Discussion

The problems associated with the assumptions required for a Vr, Wr analysis and the insensitivity of Hayman's tests for validity of the assumptions under some conditions have been reported extensively (Baker, 1978; Coughtrey and Mather, 1970; Gilbert, 1958; Sokol and Baker, 1977).

The assumptions of independent gene distribution in the parents and absence of epistasis may seem unrealistic. Failure of these assumptions may lead to overestimation of the average degree of dominance and this may have influenced the results in Table 4-9 to some extent. However, more accurate methods are unavailable at the present time and the analysis presented in Table 4-9 gives the reader a general impression of the genetic regulation of BSI in each location. The results of the combining ability analysis presented here are useful for practical plant breeders, since in both countries and elsewhere extensive effort is put into combining the smooth blossom-end scarring of lines like NC 140 and NC 8276 with other horticulturally desirable characteristics of a rougher parent in F1 hybrids.

Combining ability analysis as well as Vr, Wr analysis indicated strong additive and less important dominance effects. Implications for plant breeding are that improvements in scar size can be fixed in inbred lines and that for production of smooth hybrids both parents need to exhibit a certain amount of smoothness. NC 140 and NC 8276 had smooth blossom-end scars in both locations (Table 4-3). Both breeding lines have a pointed blossom-end morphology similar to that caused by the nipple tip (*n*) gene. The nipple tip gene has been associated with smooth blossom-end scars (Gardner and Nash, 1987). NC 140 and NC 8276 had desirable GCA in both locations. However, the SCA of hybrid combinations with NC 8276 was variable in Hazeva (Table 4-8) and thus NC 140 would be a more useful breeding line in Hazeva. In Bradenton, the performance of hybrids involving NC 140 and NC 8276 was highly predictable based on GCA and thus both lines would be useful breeding lines. Performance of the hybrid between NC 140 and NC 8276 was less than expected based on GCA, as indicated by SCA effects in the direction of larger scars in both locations (Table 4-7). However, some of the unwanted traits in NC 8276, such as beakiness (pointed fruits) and leaf curl, could be eliminated in the hybrid (R. Gardner, personal communication). It is encouraging for breeders that parent GCA is a good predictor for hybrid performance and that GCA correlates very well with the average performance of a parent.

The significant genotype x environment interaction in this experiment was investigated using Hayman and Allard analyses, and the major cause of this interaction was prominent recessive gene action in 'Suncoast' in Hazeva, which was undetected in Bradenton. This agrees with the results of Elkind et al. (1990b), who reported instability of 'Suncoast' over six locations in Israel. In the present experiment genotype x environment interaction was insignificant after elimination of 'Suncoast', in agreement with the variance component analysis reported by Elkind et al. (1990b) indicating that genotype x environment interaction was of relatively small importance. This means that the inheritance of BSI was generally the same in the two locations; however, the presence of environmentally dependent gene action as demonstrated in 'Suncoast' makes testing of F1 hybrid combinations over several locations necessary. In the present study, NC 140 and NC 8276 performed well in both locations with very different climates and are likely to perform well in a wide range of different environments. Probably only relatively rough material would be inconsistent for blossom-end scar size over locations as was the case with 'Suncoast'.

CHAPTER 5
THE INHERITANCE OF INTRA-PLANT STABILITY FOR BLOSSOM-END SCAR SIZE, CHANNELS, AND FRUIT SHAPE IN TOMATO OVER TWO ENVIRONMENTS

Introduction

Rough blossom-end scarring (RBS) of large fruited fresh market tomatoes (*Lycopersicon esculentum* Mill.) occurs to some extent in most seasons and is a serious problem in the industry. Large blossom-end scars frequently contain channels extending into the locular tissue resulting in leaking, infection by post-harvest pathogens, and thus reduced shelf life. A diallel study over two locations (Bradenton, Florida, and Hazeva, Israel) indicated that blossom-end scar size inherited primarily additively and that genotype x environment interactions were unimportant for relatively smooth genotypes (Barten et al., 1991b). NC 140 and NC 8276, both exhibiting a nippled blossom-end morphology, performed well in both locations (Barten et al., 1991b). Disadvantages of using nippling to obtain smoothness include association of nippling with genetic leaf curl and persistence of nippling resulting in beaky fruits under some conditions.

A different approach involves the use of intra-plant stability for blossom-end scar size as exhibited by 'Piedmont' and Fla 7178. These genotypes are intermediate for roughness, but most fruits within a plant have a similar scar size. These genotypes are likely to yield a higher percentage of marketable fruit than rougher types like 'Suncoast' that have a wide intra-plant variation. A preliminary experiment involving 21 cultigens indicated that the average blossom-end scar size per genotype was unrelated to the coefficient of variation in blossom-end scar size per genotype (Appendix D). Thus, the inheritance of intra-plant stability for blossom-end scar size may differ from the inheritance of average blossom-end scar size.

The purpose of this study was to investigate the inheritance of intra-plant stability as exhibited by 'Piedmont' and Fla 7178 using a generation means analysis over two locations. The usefulness of selecting for intra-plant stability when breeding for smoothness is considered. Furthermore, information was obtained on the inheritance of channels and fruit shape.

Materials and Methods

Crosses were made involving four parents differing in average scar size and intra-plant stability (Table 5-1). For the parents 'Piedmont'

Table 5-1. Subjective ratings for average scar size and intra-plant stability of parental genotypes.

Parent	Average scar size	Intra-plant stability
Suncoast	High	Low
Piedmont	Intermediate	High
Fla 7178	Intermediate	High
Fla MH-1	Low	Low

(P1) and 'Suncoast' (P2), F1, F2, and backcross generations with each parent (BCP1 and BCP2) were obtained. These six generations were grown in randomized complete block designs (RCBD) in Bradenton, Florida, and Fletcher, North Carolina, for a generation means analysis including segregating generations over two environments (Experiment 1). The four parental and three additional F1 generations, Fla 7178 x 'Suncoast', 'Piedmont' x 'Florida MH-1', and Fla 7178 x 'Florida MH-1', were also grown in a RCBD at both locations for a generation means analysis of nonsegregating generations over two environments (Experiment 2).

In Bradenton, the plants were transplanted to the field (EauGallie fine sand) on 27 February 1990 and raised under typical Florida spring weather conditions. The plants were staked, tied, and watered by seepage irrigation. Fertilizer and pesticide applications were made following recommended cultural practices. For Experiment 1, the plants were grown in eight replications each consisting of 18 5-plant plots; one plot per replication for P1, P2, and F1 generations, and five plots per replication for backcross and F2 generations. The plants for Experiment 2 were also grown in eight replications, one 5-plant plot of each genotype per replication. Windy weather caused substantial damage 2 weeks after transplanting and later high incidence of bacterial wilt incited by *Pseudomonas solanacearum* E.F. Smith resulted in reduced plant numbers.

In Fletcher, the two experiments were transplanted to the field (sandy loam) on 31 May 1990 and grown under North Carolina summer conditions following recommended cultural practices. The plants were staked, tied, and drip irrigation was used. Experiments 1 and 2 were both grown in four replications with 6-plant plots. Parental and F1 generations in both experiments consisted of one plot per replication. In Experiment 1, backcross generations consisted of five plots and the F2 generation of seven plots per replication.

Fifteen fruits were harvested per plant in both experiments from 28 May to 29 June in Bradenton and from 13 to 31 August in Fletcher. The blossom-end scar size was measured, relative to fruit size as follows:

$$\mathrm{BSI} = \frac{\sqrt{a \times b}}{\sqrt{c \times d}} \times 100$$

with BSI = blossom-end scar index,

a = largest diameter of blossom-end scar,

b = smallest diameter of blossom-end scar,

c = largest diameter of equatorial fruit cross section,

d = smallest diameter of equatorial fruit cross section.

Measurements of scar and fruit size were taken using an electronic caliper (Ultra-Cal II; Fowler-Sylvac) with a portable interface and datalogger ("1x8" Gage Talker plus; Fowler), and downloaded to a microcomputer for data analysis. For each plant the average BSI was computed as was the coefficient of variation (CV) in BSI between the fruits to measure intra-plant stability. Presence of fruit disorders such as channels, navel-like blossom-ends, zippering, and open locules (separation of the pericarp, usually along a zipper scar, exposing placental tissue) was recorded. The two parents in Experiment 1 differed in fruit shape, 'Piedmont' had flat-round fruits, whereas 'Suncoast' was deep-globe shaped. In the F2 and BCP2 generations, segregation for fruit shape was examined as well as the relationship between fruit shape and RBS.

Generation means analysis over two environments as outlined in appendix E was performed on average BSI per plant, the intra-plant CV in BSI, and the percentage of channeled fruits per plant. The spreadsheets ENVSEG.WK1 and ENVNOSEG.WK1, used for analysis of Experiments 1 and 2, respectively, required generation means and variances as input (See Appendix E). The generation means were obtained by the least squares means method (LSMEANS in SAS, Statistical Analysis System), compensating for the replication effect in the unbalanced experiments (due to plant loss, especially in Bradenton). To obtain the variance for each generation, the variance between individual plants within each plot was calculated and a weighted average over plots and replications was obtained for each generation using the degrees of freedom as weight. Calculated this way, the variance of each generation was separated from variances attributable to replication effects and generation x replication interactions.

Analysis of untransformed data indicated a relationship between means and variances of nonsegregating generations for the three characteristics studied. Individual data on average BSI and intra-plant CV in BSI were transformed using ln(x) since this transformation gave the best homogeneity of variances in nonsegregating generations. For the same reason, a $(x + 0.5)^{1/2}$ transformation was used for individual data on percentage channeled fruits (Bartlett, 1947). This rescaling affected the first degree statistics in generation means analyses only marginally.

Results

Generation Means Analysis Including Segregating Generations

The means and standard errors of transformed data for the generations in Experiment 1 are in Table 5-2. The means for F1 and F2 generations are expected to lie on the same side of the midparent value with diminishing deviations from it. Departure from this rule can only be explained by environmental and experimental variation or very large and unusual epistatic effects (Mather and Jinks, 1971). The transformed average BSI values did not follow the rule in either location (Table 5-2), and the data could only be adequately explained by a full model with complex digenic and environmental interactions (Table 5-3). The parental phenotypic distributions (Figure 5-1) showed partial overlap, and the results of Table 5-3 are probably largely influenced by environmental and experimental variation. The predominantly additive effects for average BSI in the joint scaling tests of Table 5-3 agree with the results of Barten et al. (1991b), who reported important additive effects and insignificant epistatic effects regulating average BSI in a diallel experiment involving 10 parents representing a wide array of average BSI values. Estimation of heritabilities and number of effective factors for each location would be invalid due to significance of epistatic interactions in the full model. The environmental effect (e) on average BSI was not significant (Table 5-3).

For intra-plant stability in BSI, the additive-dominance model with environment interaction was not rejected at $\alpha = 0.01$ ('CV in BSI')

Table 5-2. Generation means and standard errors for transformed values of average blossom-end scar index (BSI) per plant, intra-plant coefficient of variation (CV) in BSI, and percentage channeled fruits per plant in the 'Piedmont' x 'Suncoast' generations at Bradenton (B) and Fletcher (F).

Generation	Location	Plant no.	Average BSI ln(x)		CV in BSI ln(x)		% channeled $(x + 0.5)^{1/2}$	
			Mean	Std Err	Mean	Std Err	Mean	Std Err
P1 (Piedmont)	B	34	2.222	0.018	3.459	0.045	4.007	0.327
BCP1	B	137	2.233	0.014	3.463	0.020	4.813	0.153
F1	B	35	2.348	0.025	3.491	0.040	5.851	0.259
F2	B	181	2.283	0.014	3.515	0.019	5.607	0.127
Midparent[z]	B	---	2.334	0.019	3.517	0.034	5.477	0.201
BCP2	B	159	2.406	0.014	3.615	0.022	6.644	0.117
P2 (Suncoast)	B	33	2.445	0.034	3.575	0.050	6.947	0.232
P1 (Piedmont)	F	23	2.120	0.023	3.301	0.057	3.676	0.287
BCP1	F	120	2.162	0.013	3.315	0.023	4.116	0.194
F1	F	24	2.258	0.022	3.555	0.050	5.281	0.229
F2	F	166	2.234	0.016	3.524	0.023	5.269	0.153
Midparent[z]	F	---	2.305	0.019	3.505	0.035	4.918	0.191
BCP2	F	119	2.411	0.014	3.636	0.025	5.937	0.133
P2 (Suncoast)	F	23	2.489	0.029	3.708	0.040	6.160	0.252

[z] Estimated as $0.5 \cdot (\overline{P1} + \overline{P2})$, with standard error $[0.25 \cdot \{Var(P1)/n_{P1} + Var(P2)/n_{P2}\}]^{1/2}$

Table 5-3. Joint scaling tests and perfect fit estimates for average blossom-end scar index (BSI) per plant, intra-plant coefficient of variation (CV) in BSI, and percentage channeled fruits per plant in 'Piedmont' x 'Suncoast' generations at Bradenton and Fletcher.

Parameter^{z}	Average BSI ln(x)		CV in BSI ln(x)		% Channeled $(x + 0.5)^{1/2}$	
	Estimate	Std Err	Estimate	Std Err	Estimate	Std Err
Additive-dominance model						
m	2.322	0.026***^{y}	3.518	0.043***	5.196	0.118***
[d]	-0.174	0.023***	-0.181	0.036***	-1.568	0.103***
[h]	-0.051	0.050^{NS}	-0.012	0.084^{NS}	0.429	0.220^{NS}
e^{w}	0.023	0.012^{NS}	0.016	0.018^{NS}	0.287	0.052***
χ^2	49.124		39.217		8.488	
P value	<0.001		<0.001		0.387	
Additive-dominance model with environment interaction						
m	2.316	0.024***^{y}	3.503	0.032***	5.190	0.127***
[d]	-0.174	0.021***	-0.189	0.026***	-1.562	0.111***
[h]	-0.041	0.046^{NS}	0.015	0.062^{NS}	0.442	0.237^{NS}
e	0.013	0.024^{NS}	0.035	0.032^{NS}	0.260	0.127^{NS}
Gde	0.041	0.021^{NS}	0.078	0.026*	-0.107	0.111^{NS}
Ghe	0.016	0.046^{NS}	-0.040	0.062^{NS}	0.033	0.237^{NS}
χ^2	30.272		15.713		7.354	
P value	<0.001		0.015		0.289	
Model including digenic interactions						
m	2.147	0.097***^{y}	3.554	0.192***	5.319	0.390***
[d]	-0.147	0.025**	-0.132	0.058^{NS}	-1.352	0.105***
[h]	0.291	0.236^{NS}	-0.111	0.482^{NS}	0.139	0.968^{NS}
[i]	0.179	0.094^{NS}	-0.027	0.183^{NS}	-0.108	0.375^{NS}
[j]	-0.129	0.072^{NS}	-0.184	0.161^{NS}	-0.938	0.309*
[l]	-0.138	0.153^{NS}	0.070	0.329^{NS}	0.109	0.640^{NS}
e	0.023	0.009^{NS}	0.015	0.020^{NS}	0.291	0.039***
χ^2	18.086		30.838		2.902	
P value	0.003		<0.001		0.715	
Full model including digenic and environment interactions						
m	2.141	0.051***^{x}	3.560	0.079***	5.439	0.521***
[d]	-0.148	0.013***	-0.131	0.024***	-1.356	0.138***
[h]	0.308	0.124*	-0.124	0.197^{NS}	-0.130	1.293^{NS}
[i]	0.178	0.050***	-0.049	0.075^{NS}	-0.241	0.502^{NS}
[j]	-0.126	0.038**	-0.212	0.066**	-0.941	0.411*
[l]	-0.146	0.081^{NS}	0.087	0.134^{NS}	0.258	0.852^{NS}
e	0.047	0.051^{NS}	-0.139	0.079^{NS}	-0.448	0.521^{NS}
Gde	0.037	0.013**	0.073	0.024**	-0.114	0.138^{NS}
Ghe	-0.087	0.124^{NS}	0.431	0.197*	1.736	1.293^{NS}
Gie	-0.032	0.050^{NS}	0.145	0.075^{NS}	0.728	0.502^{NS}
Gje	0.004	0.038^{NS}	0.024	0.066^{NS}	0.219	0.411^{NS}
Gle	0.085	0.081^{NS}	-0.324	0.134*	-1.003	0.852^{NS}

---

[z] See appendix E for explanation of parameters.

[y,x] NS,*,**,***: Not significant or significant at $\alpha = 0.05$, $\alpha = 0.01$, or $\alpha = 0.001$ in Student's t tests (y) and a t' test (x).

[w] The environmental parameter is +e for Bradenton and -e for Fletcher.

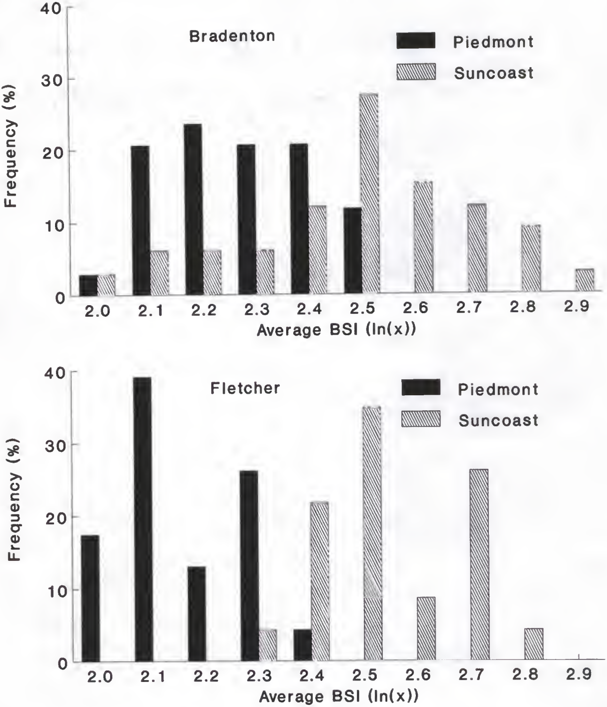

Figure 5-1. Frequency distribution of transformed average blossom-end scar index (BSI) for 'Piedmont' and 'Suncoast' in Bradenton and Fletcher. The maximum value in each class is indicated on the X-axis.

in Table 5-3). Intra-plant stability was inherited mainly additively and the interaction between [d] and e, represented as Gde, was significant at $\alpha = 0.05$ (Table 5-3). Analysis per location (Table 5-4) showed that additive effects were of greater magnitude in Fletcher than in Bradenton which was also apparent from the differences between

Table 5-4. Joint scaling tests for the intra-plant coefficient of variation (CV) in blossom-end scar index (BSI) and percentage channeled fruits per plant in the 'Piedmont' x 'Suncoast' generations at Bradenton and Fletcher.

Parameter[z]	CV in BSI ln(x)		% Channeled $(x + 0.5)^{1/2}$	
	Estimate	Std Err	Estimate	Std Err
		Bradenton		
m	3.538	0.038^{***y}	5.451	0.155^{***}
[d]	-0.111	0.031^{*}	-1.669	0.130^{**}
[h]	-0.025	0.072^{NS}	0.474	0.294^{NS}
X^2	5.879		2.661	
P value	0.118		0.117	
B.S.[x]	-0.02		0.12	
N.S.[w]	0.09		0.17	
k[v]	0.29		2.21	
		Fletcher		
m	3.469	0.052^{***y}	4.930	0.201^{***}
[d]	-0.268	0.043^{**}	-1.455	0.183^{**}
[h]	0.054	0.102^{NS}	0.409	0.367^{NS}
X^2	9.834		4.693	
P value	0.020		0.196	
B.S.[x]	0.34		0.61	
N.S.[w]	0.38		0.30	
k[v]	0.64		0.65	

[z] See appendix E for explanation of parameters.

[y] NS,*,**,***: Not significant or significant at $\alpha = 0.05$, $\alpha = 0.01$, or $\alpha = 0.001$ in Student's t test.

[x] B.S. = Broad Sense heritability, estimated according to Allard (1960); B.S. = [Var(F2) - {Var(P1) + Var(P2) + Var(F1)}/3]/Var(F2).

[w] N.S. = Narrow Sense heritability, estimated according to Warner (1952); N.S. = {2·Var(F2) - Var(BCP1) - Var(BCP2)}/Var(F2).

[v] k = number of effective factors, estimated according to the formula in appendix E.

parents in Bradenton and Fletcher in Table 5-2. In each location the additive-dominance model was adequate at $\alpha = 0.01$, and broad sense (Allard, 1960) and narrow sense (Warner, 1952) heritability estimates could be obtained (Table 5-4). The heritability for intra-plant stability was greater in Fletcher than in Bradenton, mostly due to wider genetic variation in Fletcher (Table 5-2). The estimated number of effective factors (k) was low in both locations (Table 5-4). Underestimation of k can result from unequal gene-effects or a non-isodirectional distribution of alleles over the parents (Mather and Jinks, 1971). The data presented in Table 5-4 indicated that intra-plant stability was regulated by a few (probably one or two) genetic factors and largely influenced by the environment. The conclusions from the generation means analysis on intra-plant stability need to be considered with some reservation since the parental phenotype distributions overlap, especially in Bradenton (Figure 5-2).

For the percentage channeled fruits per plant, an additive-dominance model without environmental interactions proved appropriate in the joint scaling test (Table 5-3). The occurrence of channels inherited mainly additively with a highly significant environmental effect (Table 5-3). The average percentage channeled fruits per plant was higher in Bradenton than in Fletcher (Table 5-4). In spite of the greater expression, heritability values in Bradenton were lower than in Fletcher (Table 5-4) which was probably due to high non-heritable variation in Bradenton as compared to Fletcher. Non-heritable variation, estimated as [Var(P1) + Var(P2) + Var(F1)]/3, was 2.59 and 1.54 for the percentage channeled fruits per plant in Bradenton and

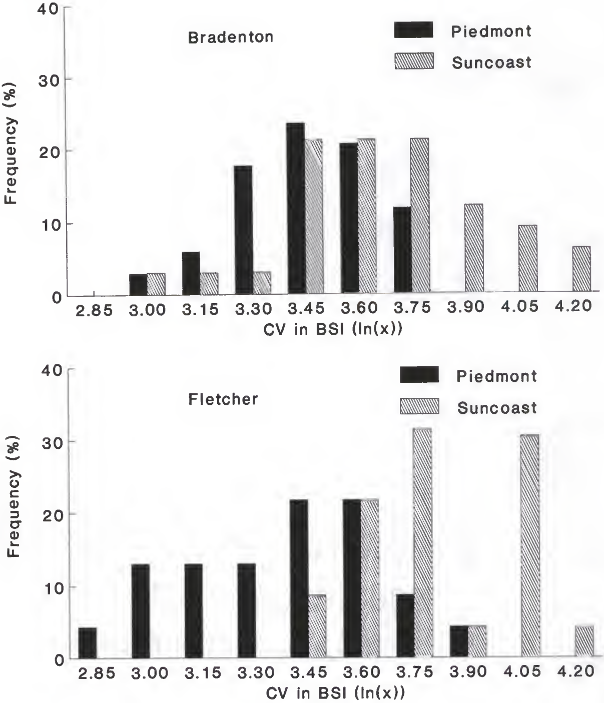

Figure 5-2. Frequency distribution of transformed intra-plant coefficient of variation (CV) in average blossom-end scar index (BSI) for 'Piedmont' and 'Suncoast' in Bradenton and Fletcher. The maximum value in each class is indicated on the X-axis.

Fletcher, respectively. The estimated number of effective factors differed between Bradenton and Fletcher (Table 5-4), and indicated that at least several factors must be influencing the percentage channeled fruits per plant. The parental phenotype distributions showed partial overlap (Figure 5-3).

Generation Means Analysis of Nonsegregating Generations

The generation means and standard errors for transformed data of the generations in Experiment 2 are reported in Table 5-5. For generation means analysis of nonsegregating generations over two locations epistatic effects were assumed insignificant. The inheritance of average BSI per plant was different for each cross (Table 5-6). For Fla 7178 x 'Suncoast' and Fla 7178 x 'Florida MH-1', the additive-dominance model without environment interaction was appropriate at α = 0.01. In the former cross only additive effects were significant, whereas in the latter dominance effects were significant as well (Table 5-6). In the cross 'Piedmont' x 'Florida MH-1', the additive effects interacted significantly with location and dominance effects were highly significant in the full model (Table 5-6). For this cross, the additive effects, estimated as $0.5 \cdot (\overline{P1} - \overline{P2})$, were greater in Bradenton than in Fletcher (Table 5-5). Environmental effects on average BSI were insignificant in all three crosses (Table 5-6). The parental phenotype distributions for average BSI showed overlap for each cross (data not shown).

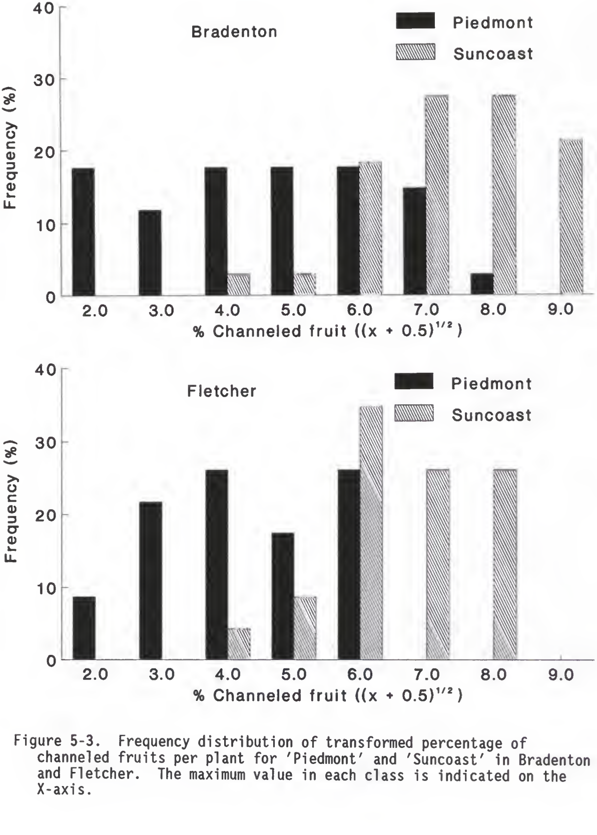

Figure 5-3. Frequency distribution of transformed percentage of channeled fruits per plant for 'Piedmont' and 'Suncoast' in Bradenton and Fletcher. The maximum value in each class is indicated on the X-axis.

Table 5-5. Generation means and standard errors for transformed values of average blossom-end scar index (BSI) per plant, intra-plant coefficient of variation (CV) in BSI, and percentage channeled fruits per plant in four parental and three F1 generations at Bradenton (B) and Fletcher (F).

Generation	Location	Plant no.	Average BSI ln(x)		CV in BSI ln(x)		% channeled $(x + 0.5)^{\frac{1}{2}}$	
			Mean	Std Err	Mean	Std Err	Mean	Std Err
Parents								
Suncoast	B	31	2.574	0.029	3.640	0.038	7.096	0.264
Piedmont	B	35	2.249	0.028	3.461	0.038	4.484	0.358
Fla 7178	B	38	2.218	0.015	3.475	0.041	4.309	0.221
Fla MH-1	B	35	1.684	0.025	3.539	0.065	4.173	0.292
F1's								
Fla 7178 x Suncoast	B	40	2.373	0.025	3.553	0.039	5.378	0.283
Piedmond x Fla MH-1	B	37	2.151	0.029	3.653	0.043	6.122	0.286
Fla 7178 x Fla MH-1	B	39	2.164	0.025	3.533	0.035	6.044	0.308
Parents								
Suncoast	F	24	2.566	0.036	3.856	0.055	6.614	0.177
Piedmont	F	24	2.101	0.020	3.363	0.053	4.797	0.260
Fla 7178	F	23	2.234	0.019	3.383	0.063	3.732	0.224
Fla MH-1	F	24	1.809	0.040	3.845	0.081	5.202	0.196
F1's								
Fla 7178 x Suncoast	F	23	2.276	0.025	3.564	0.042	4.606	0.249
Piedmont x Fla MH-1	F	23	2.083	0.022	3.520	0.057	6.775	0.193
Fla 7178 x Fla MH-1	F	24	2.205	0.025	3.594	0.062	6.696	0.189

Table 5-6. Joint scaling tests and perfect fit estimates for average blossom-end scar index (BSI) per plant transformed to ln(x) in nonsegregating generations of three crosses at Bradenton and Fletcher.

Parameter[z]	Fla 7178 x Suncoast		Piedmont x Fla MH-1		Fla 7178 x Fla MH-1	
	Estimate	Std Err	Estimate	Std Err	Estimate	Std Err
			Additive-dominance model			
m	2.396	0.024***[y]	1.935	0.045***	1.979	0.018***
[d]	-0.174	0.024*	0.227	0.047*	0.250	0.018**
[h]	-0.070	0.041^{NS}	0.181	0.075^{NS}	0.206	0.032*
e[w]	0.009	0.017^{NS}	0.029	0.037^{NS}	-0.020	0.014^{NS}
χ^2	7.002		22.261		4.257	
P value	0.030		<0.001		0.119	
			Additive-dominance model with environment interaction			
m	2.398	0.013***[x]	1.961	0.014***	1.986	0.013***
[d]	-0.172	0.013***	0.214	0.014***	0.240	0.013***
[h]	-0.074	0.022**	0.156	0.023***	0.198	0.022***
e	-0.002	0.013^{NS}	0.006	0.014^{NS}	-0.035	0.013**
Gde	-0.006	0.013^{NS}	0.068	0.014***	0.027	0.013*
Ghe	0.051	0.022*	0.028	0.023^{NS}	0.015	0.022^{NS}

[z] See appendix E for explanation of parameters.

[y,x] NS,*,**,***: Not significant or significant at $\alpha = 0.05$, $\alpha = 0.01$, or $\alpha = 0.001$ in Student's t test (y) and a t′ test (x).

[w] The environmental parameter is +e for Bradenton and -e for Fletcher.

The results of generation means analysis of intra-plant stability for nonsegregating generations were similar for the three crosses in Table 5-7 and agreed with the analysis including segregating generations for 'Piedmont' x 'Suncoast' (Table 5-3). The additive-dominance model was inadequate at $\alpha = 0.01$ for all crosses (Table 5-7). The additive effects interacted significantly with locations, and for all crosses the magnitude of [d] was greater in Fletcher than in Bradenton (Table 5-5). The increase in additive effects in Fletcher was the result of a slight increase in stability of the stable parents (low CV in BSI), combined with a dramatic decrease in stability of the unstable parents (high CV in BSI) as compared to Bradenton (Table 5-5). For 'Piedmont' x 'Florida

Table 5-7. Joint scaling tests and perfect fit estimates for intra-plant coefficient of variation in blossom-end scar index transformed to ln(x) in nonsegregating generations of three crosses at Bradenton and Fletcher.

Para-	Fla 7178 x Suncoast		Piedmont x Fla MH-1		Fla 7178 x Fla MH-1	
meter[z]	Estimate	Std Err	Estimate	Std Err	Estimate	Std Err
			Additive-dominance model			
m	3.588	0.055***[y]	3.538	0.079***	3.562	0.069***
[d]	-0.130	0.052^{NS}	-0.117	0.078^{NS}	-0.103	0.067^{NS}
[h]	-0.029	0.085^{NS}	0.061	0.119^{NS}	-0.001	0.096^{NS}
e[w]	-0.025	0.043^{NS}	0.019	0.057^{NS}	-0.026	0.051^{NS}
X^2	10.325		13.680		9.761	
P value	0.006		0.001		0.008	
			Additive-dominance model with environment interaction			
m	3.589	0.025***[x]	3.552	0.031***	3.561	0.032***
[d]	-0.159	0.025***	-0.140	0.031***	-0.132	0.032***
[h]	-0.030	0.038^{NS}	0.034	0.047^{NS}	0.003	0.048^{NS}
e	-0.031	0.025^{NS}	-0.052	0.031^{NS}	-0.054	0.032^{NS}
Gde	0.077	0.025**	0.101	0.031**	0.099	0.032***
Ghe	0.026	0.038^{NS}	0.119	0.047*	0.023	0.048^{NS}

[z] See appendix E for explanation of parameters.

[y,x] NS,*,**,***: Not significant or significant at $\alpha = 0.05$, $\alpha = 0.01$, or $\alpha = 0.001$ in Student's t test (y) and a t′ test (x).

[w] The environmental parameter is +e for Bradenton and -e for Fletcher.

MH-1', the dominance effect interacted significantly ($\alpha = 0.05$) with locations (Table 5-7). The parental phenotype distributions showed overlap in both locations, but the separation between the parents was better in Fletcher than in Bradenton (data not shown).

The difference in average percentage channeled fruits per plant between parents was significant at both locations only for the cross Fla 7178 x 'Suncoast' (Table 5-5). Generation means analysis for this cross indicated adequacy of the additive-dominance model with significant additive, dominance, and environmental effects (Table 5-8). The significant positive value for e indicates that the occurrence of

Table 5-8. Joint scaling test for percentage channeled fruits per plant transformed to $(x + 0.5)^{1/2}$ in nonsegregating generations of Fla 7178 x 'Suncoast' at Bradenton and Fletcher.

Parameter[z]	Estimate	Std Err
	Additive-dominance model	
m	5.448	0.046***[y]
[d]	-1.428	0.046**
[h]	-0.467	0.091*
e[x]	0.296	0.040*
X^2	0.353	
P value	0.838	

[z] See appendix E for explanation of parameters.

[y] *,**,***: Significant at $\alpha = 0.05$, $\alpha = 0.01$, or $\alpha = 0.001$ in Student's t test.

[x] The environmental parameter is +e for Bradenton and -e for Fletcher.

channels was greater in Bradenton than in Fletcher for the cross Fla 7178 x 'Suncoast'.

Intra-plant Stability and Marketability

In the crosses 'Piedmont' x 'Suncoast' and Fla 7178 x 'Suncoast', hybrid performance for average BSI, intra-plant CV in BSI, and percentage unmarketable fruits (scar length > 1 cm, Wien and Zhang, 1991) was close to midparent values, indicating mostly additive inheritance in both locations (Table 5-9). The relative importance of intra-plant CV in BSI on marketability was hard to separate from average BSI since both characteristics inherited additively and for the parents in these crosses high values for BSI coincided with high values for

Table 5-9. Percentage unmarketable fruits in four hybrid combinations in relation to average blossom-end scar index (BSI) and average intra-plant coefficient of variation (CV) in BSI.

Generation	Bradenton				Fletcher			
	Plant no.	Average BSI	CV in BSI	% Unmarketable	Plant no.	Average BSI	CV in BSI	% Unmarketable
Experiment 1								
Piedmont	34	9.31	32.67	24.2	23	8.39	28.05	19.5
Suncoast	33	11.81	37.13	43.8	23	12.17	41.66	56.3
Piedmont x Suncoast	35	10.60	33.65	35.8	24	9.62	36.40	39.6
Experiment 2								
Fla 7178	38	9.23	33.31	25.8	23	9.39	30.76	38.0
Suncoast	31	13.35	38.97	53.0	24	13.19	48.85	61.1
Fla 7178 x Suncoast	40	10.87	36.06	36.3	23	9.82	36.12	45.3
Piedmont	35	9.61	32.55	28.6	24	8.22	29.77	21.1
Fla MH-1	35	5.45	36.42	3.3	24	6.23	50.74	10.9
Piedmont x Fla MH-1	37	8.71	39.94	21.6	23	8.08	35.12	19.2
Fla 7178	38	9.23	33.31	25.8	23	9.39	30.76	38.0
Fla MH-1	35	5.45	36.42	3.3	24	6.23	50.74	10.9
Fla 7178 x Fla MH-1	39	8.81	35.02	19.4	24	9.14	37.87	30.0

intra-plant CV in BSI (Table 5-9). However, 'Florida MH-1' had a low average BSI and a high intra-plant CV in BSI. Hybrid performance for average BSI and marketability in 'Piedmont' x 'Florida MH-1' and Fla 7178 x 'Florida MH-1' indicated the presence of dominance in the direction of higher average BSI and lower marketability (Table 5-9). This agreed with significant positive estimates for [h] in the joint scaling test for Fla 7178 x 'Florida MH-1' and in the full model for 'Piedmont' x 'Florida MH-1' in Table 5-6. In Fletcher, where the average intra-plant CV in BSI for 'Florida MH-1' was much higher than in Bradenton, the hybrid performance for average BSI and marketability was very close to the least desirable parent in the crosses involving 'Florida MH-1' (Table 5-9). High intra-plant variation in 'Florida MH-1' probably resulted in higher average BSI values in the hybrid than expected based on additive inheritance. Thus, for good hybrid performance low intra-plant variation is needed in both parents as well as low average BSI.

An approach to investigate the relative importance of intra-plant stability in BSI for marketability involved further investigation of the F2 generations of 'Piedmont' x 'Suncoast' in Bradenton and Fletcher. For F2 plants, the correlation of average BSI with intra-plant CV in BSI was significant ($P < 0.001$) but low, the Pearson correlation coefficients being 0.35 and 0.31 in Bradenton and Fletcher, respectively. This means that average BSI and intra-plant stability for BSI were inherited largely independently of each other. For groups of F2 individuals with similar average BSI, the correlation between intra-plant CV in BSI and percentage unmarketable fruits per plant was

Table 5-10. Pearson correlation coefficients (r) between intra-plant coefficient of variation in blossom-end scar index (BSI) and percentage unmarketable fruits in the F2 generation of 'Piedmont' x 'Suncoast', grouped according to average BSI.

Average BSI	Bradenton			Fletcher		
	Plant no.	r	*P* value	Plant no.	r	*P* value
< 8	26	0.45	0.021	39	0.30	0.063
8 - 9	34	0.30	0.089	33	0.06	0.735
9 - 10	42	-0.24	0.128	39	-0.17	0.295
10 - 11	26	-0.40	0.040	27	-0.48	0.012
11 >	53	-0.10	0.457	28	-0.48	0.010

calculated for both locations (Table 5-10). Only in Bradenton when the average BSI was low (< 8), did higher intra-plant stability (lower CV in BSI) result in a lower percentage unmarketable fruits ($\alpha = 0.05$; Table 5-10). Thus the direct influence of intra-plant stability on marketability was relatively unimportant.

Fruit Shape and Disorders

In the F2 generation of 'Piedmont' x 'Suncoast', the percentage of fruits exhibiting channels, navels, zippers, or open locules was calculated per plant and correlated with average BSI per plant (Table 5-11). The correlation between average BSI and percentage channeled fruits was high and highly significant in both locations. Thus plants with larger scars are more likely to have fruits that contain channels. Fortney (1958) reported correlation coefficients between presence of stylar scar openings and scar size varying from 0.55 to 0.72. Fruits with navel-like blossom-ends generally had larger scars, resulting in a

Table 5-11. Pearson correlation coefficients and *P* values for percentage fruits with disorders per plant and average blossom-end scar index (BSI) per plant in the F2 generation of 'Piedmont' x 'Suncoast' in Bradenton (n = 181, above diagonal) and Fletcher (n = 166, below diagonal).

	Average BSI	% Channels	% Navels	% Zippers	% Open locules
Average BSI	---	0.73 <0.001	0.53 <0.001	0.08 0.260	-0.01 0.870
% Channels	0.72 <0.001	---	0.42 <0.001	0.07 0.367	0.08 0.306
% Navels	0.65 <0.001	0.43 <0.001	---	-0.05 0.514	-0.04 0.625
% Zippers	-0.07 0.385	0.05 0.530	-0.08 0.282	---	0.33 <0.001
% Open locules	-0.10 0.204	-0.05 0.484	-0.06 0.441	0.65 <0.001	---

significant positive correlation between percentage fruits with navel-like blossom-ends and average BSI (Table 5-11). Zippers and open locules occurred independent of scar size and channeling, but a significant correlation existed between the percentage fruits with zippering and the percentage fruits in which the zippers opened up, resulting in open locules (Table 5-11).

The fruit shape in F2 and BCP2 generations could be classified in two categories; flat-round and deep-globe. The data were fitted to a single gene model with complete dominance for flat-round shape (Table 5-12). In the BCP2 generation in Bradenton, the observed segregation deviated significantly from the expected ratio, whereas in the F2 generation the observed values fitted closely to the expected 3:1 segregation. This tendency was more pronounced when pooling the data over the two locations (Table 5-12). A two gene model with duplicate dominance was not a feasible alternative explanation since deviation

Table 5-12. Segregation for fruit shape (flat-round = f-r v.s. deep-globe = d-g) in 'Piedmont' (P) x 'Suncoast' (S) generations in Bradenton and Fletcher.

Generation	Genotype	Observed		Expected		X^2	*P* value
		f-r	d-g	f-r	d-g		
Bradenton							
P1	P	34	0	34	0	--	--
BCP1	Px(PxS)	139	0	139	0	--	--
F1	PxS	35	0	35	0	--	--
F2	PxS	148	47	146.25	48.75	0.08	0.772
BCP2	Sx(PxS)	103	68	85.5	85.5	7.16	0.007
P2	S	0	36	0	36	--	--
Fletcher							
P1	P	23	0	23	0	--	--
BCP1	Px(PxS)	120	0	120	0	--	--
F1	PxS	24	0	24	0	--	--
F2	PxS	135	33	126	42	2.57	0.109
BCP2	Sx(PxS)	70	49	59.5	59.5	3.71	0.054
P2	S	0	24	0	24	--	--
Pooled							
F2	PxS	283	80	272.25	90.75	1.70	0.193
BCP2	Sx(PxS)	173	117	145	145	10.81	0.001

from 15:1 and 3:1 was highly significant in both locations in the F2 and BCP2 generations, respectively. All plants exhibiting a deep-globe fruit shape had a long pedicel similar to 'Suncoast' as opposed to the short pedicel characteristic for 'Piedmont'. This indicated either a strong linkage between two different genes or, more likely, pleiotrophy since no recombination occurred in 290 BCP2 and 363 F2 individuals.

In generations segregating for fruit shape, the relationship between fruit shape and BSI as well as occurrence of fruit malformations was examined. Data for each fruit characteristic were averaged separately for flat-round and deep-globe fruited plants (Table 5-13).

Table 5-13. Significance of the difference between flat-round (f-r) and deep-globe (d-g) fruited plants for several fruit characteristics in BCP2 and F2 generations of 'Piedmont' (P1) x 'Suncoast' (P2) in Bradenton and Fletcher.

	Bradenton				Fletcher			
	BCP2		F2		BCP2		F2	
	f-r	d-g	f-r	d-g	f-r	d-g	f-r	d-g
Number of plants	95	62	133	43	70	49	133	33
Average BSI	11.4	$11.0^{NS,z}$	10.2	9.5^{*z}	11.6	10.9^{*y}	9.7	8.8^{***y}
% Channels	49.3	41.1^{*z}	37.3	26.1^{***z}	42.9	28.1^{***y}	34.7	17.3^{***y}
% Navels	7.7	3.4^{***y}	4.5	1.4^{***y}	5.9	1.5^{***y}	3.8	0.4^{***y}
% Zippers	1.0	$1.4^{NS,z}$	1.7	$2.0^{NS,z}$	1.2	$1.5^{NS,z}$	1.6	$0.8^{NS,y}$
% Open locules	0.2	$0.2^{NS,z}$	0.2	$0.3^{NS,z}$	0.5	$1.0^{NS,y}$	0.5	$0.6^{NS,z}$

[z,y] NS,*,**,***: Differences not significant or significant at $\alpha = 0.05$, $\alpha = 0.01$, or $\alpha = 0.001$ in Student's t tests (z) and t' tests (y).

The two means with unequal sample size were compared by use of Student's t test if the sample variances were not significantly different and a t' test otherwise (Steel and Torrie, 1980; Table 5-13). The average BSI was significantly higher in flat-round than in deep-globe fruited plants in all segregating generations except the BCP2 generation in Bradenton. However, differences were small and average BSI was high for both fruit shapes (Table 5-13). The occurrence of channels and navel-like blossom-ends was significantly higher in flat-round fruited plants as compared to deep-globe fruited ones. No significant difference existed between the fruit shapes for occurrence of zippers and open locules (Table 5-13). These results agree with Fortney (1958) who reported increased scar size and increased occurrence of stylar scar openings with increased flatness of the fruit ($r = 0.43 - 0.47$ and $r = 0.31 - 0.37$, respectively).

Discussion

The crosses for this experiment were aimed at maximizing the difference in intra-plant CV in BSI rather than average BSI between the parents (Table 5-5). Relatively small differences between parents in average BSI lead to problems with environmental and experimental variation in the generation means analysis of average BSI in Experiment 1 (Table 5-3). However, reliable information on the inheritance of average BSI is available from a 10 parent diallel experiment over two locations, Bradenton, Florida, and Hazeva, Israel (Barten et al., 1991b). In this diallel experiment epistatic effects were insignificant, additive and dominance effects were both significant, but the additive effects were more important. In the present experiment, additive effects were significant for average BSI in each of the four crosses (Tables 5-2 and 5-6). In addition, significant dominance effects in the direction of larger BSI regulated the inheritance of average BSI in the crosses involving 'Florida MH-1' as a parent (Table 5-6). In the diallel experiment, however, the dominance effects were negative (in the direction of smaller scars) when summed over all loci (Barten et al., 1991b), probably due to negative dominance in other crosses in the diallel. The interaction of additive and dominance effects with environment in the diallel experiment was due to recessive gene action in 'Suncoast' in Hazeva. After exclusion of 'Suncoast' from the diallel analysis, genotype x environment interaction was insignificant (Barten et al., 1991b). In the present experiment, however, the genotype x environment interaction was insignificant in Fla 7178 x 'Suncoast' and the average BSI for 'Suncoast' was nearly identical in Bradenton and Fletcher. Fla 7178 was not included in the diallel.

The inheritance of intra-plant stability was additive (Tables 5-3 and 5-7), which means that high intra-plant stability is necessary in both parents to produce a stable hybrid. Furthermore, low heritability estimates in both locations (Table 5-4) indicate that breeding for improvement of intra-plant stability would be difficult. This information, combined with the fact that the influence of intra-plant stability on marketability is relatively unimportant compared to the influence of average BSI (Table 5-10), makes breeding for intra-plant stability an undesirable approach. An important aspect of intra-plant stability is the relationship between instability in 'Florida MH-1' and dominance towards high average BSI in hybrids involving 'Florida MH-1' (Tables 5-6 and 5-9). Such a relationship was not evident for 'Suncoast' (Table 5-9). However, in the diallel experiment mentioned before, 'Suncoast' exhibited unexpectedly high BSI values in Hazeva, and additive as well as dominance effects interacted significantly with the environment in crosses involving 'Suncoast'. Thus, 'Florida MH-1' and 'Suncoast', both characteristically unstable for BSI, exhibited unpredictable hybrid performance for average BSI in different locations. Strict selection against genotypes with high intra-plant variation in BSI seems advisable in a hybrid breeding program to prevent unpredictable hybrid performance or instability over locations. Selection against high intra-plant variation would have been more efficient in Fletcher than in Bradenton, since differences between genotypes were more pronounced and especially unstable genotypes were easier identified in Fletcher (Table 5-5).

The percentage channeled fruits per plant inherited additively in 'Piedmont' x 'Suncoast' and Fla 7178 x 'Suncoast', and narrow sense heritability in the former cross was estimated at 0.17 and 0.30 in Bradenton and Fletcher, respectively (Table 5-4). Fortney (1958) also reported additive inheritance for the frequency of scar openings with heritability estimates of 0.25 for 'Rutgers' x Purdue 7266 and 0.55 for 'Golden Jubilee' x 'Kokomo'. The presence of channels plays a major role in the occurrence of fruit rot and is thus undesirable (Fortney, 1958). However, selection against channels is difficult under field situations since close inspection of the blossom-end is needed. Selection based on blossom-end scar size is easier in field situations and seems a feasible approach since indirect selection against the occurrence of channels can be expected based on the high correlation between prevalence of channels and average BSI per plant (Table 5-11). In the diallel experiment, the narrow sense heritability for average BSI, estimated according to Mather and Jinks (1971), was 0.61 in Bradenton (Barten et al., 1991b).

Based on the results of Table 5-12, it is difficult to reach a conclusion on inheritance of fruit shape without more extensive experimentation. Several alternative explanations are possible for the current data. The difference between F2 and BCP2 generations in deviation from expected ratios may be the result of a cytoplasmatic factor in 'Suncoast' causing an underrepresentation of deep-globe fruit shapes in the BCP2 generations. A reciprocal F2 generation is needed to substantiate this theory. Alternatively, post-syngamic selection against deep-globe shaped plants is a possible explanation of the results. However, in a separate experiment, seed germination in the BCP2 and F2 generations (respectively 53.7% and 49.0%) was better than in the BCP1 generation (44.4%). Yet another hypothesis is that the data in Table 5-12 are the result of preferential fertilization. In tomato, the microgametophytic factor *X* causes abortion of microgametophytes carrying the recessive allele *x* in heterozygous (*Xx*) genotypes (Alexander, 1973). In the F2 and BCP2 generations of Table 5-12, heterozygous genotypes served as the male parent and linkage of deep-globe fruit shape with *x* in the coupling phase may be the cause of underrepresentation of the deep-globe fruit shape in segregating generations. However, the deviations from expected segregations in Table 5-12 are not as pronounced as those reported for the *I/i* (Kedar et al., 1967) and *J/j* (Vriesenga and Honma, 1971) loci, both closely linked to the *X/x* locus. The relatively high number of deep-globe shaped plants in Table 5-12 can be explained by crossing over between the fruit shape locus and the *X/x* locus and by incomplete penetrance of *X*. Assuming a penetrance of 96% for *X* (Alexander, 1973), the data in Table 5-12 can be explained by 39% and 40% crossing over in the BCP2 generations, and 48% and 38% crossing over in the F2 generations in Bradenton and Fletcher, respectively. The difference between F2 and BCP2 generations in deficiency of deep-globe shaped plants may be explained by unequal efficiency of preferential fertilization, depending on the female genotype (Bemis, 1959). The preferential fertilization would then be more efficient in a deep-globe shaped female as compared to a heterozygous, flat-round shaped female. Reciprocal backcross generations are needed to differentiate between post-syngamic selection and preferential fertilization (Kedar et al., 1967). A study of the possible linkage between *I/i*, *J/j*, and the fruit shape locus may be useful in this respect as well as observations on *in vivo* pollen germination and tube growth.

CHAPTER 6

THE CHARACTERIZATION OF BLOSSOM-END MORPHOLOGY GENES IN TOMATO AND THEIR USEFULNESS IN BREEDING FOR SMOOTH BLOSSOM-END SCARS

Introduction

Rough blossom-end scarring (RBS) is a recurring problem in large fruited fresh market tomatoes (*Lycopersicon esculentum* Mill.). Tomato breeders frequently use genotypes with a pointed blossom-end morphology to obtain smooth blossom-end scars. Unfortunately, this pointed blossom-end character is generally associated with leaf curling that often results in increased foliar disease problems (Gardner and Nash, 1987). Usually, the pointed blossom-end morphology is most pronounced in young fruits and disappears as the fruit develops. However, under some conditions pointed blossom-ends persist on mature fruit, leading to post-harvest handling problems. The problems with leaf curling and variable expression of persistent pointedness prompted the investigation of previously described blossom-end morphology genes and a search for new sources in an attempt to obtain alternatives to the currently available blossom-end morphology genes.

Several blossom-end morphology genes have been described. The nipple tip (*n*) allele (MacArthur, 1934) produces a nipple-like protuberance on the blossom-end and has been assigned to chromosome 5 (Mutschler et al., 1987). Single recessive gene action was suggested by F2 segregations of 3 normal to 1 nipple tip (Young and MacArthur, 1947); however, Tikoo and Anand (1984) reported that nipple tip in their material was controlled by two different genes. The beaked fruit (*bk*) allele (Young and MacArthur, 1947) is characterized by a prominent pointed beak on the blossom-end and has been located on chromosome 2 (Mutschler et al., 1987). Bouwkamp and Honma (1970) reported a second, dominant allele (*Bk*-2) associated with beaked fruits. In fruits with the persistent style (*pst*) allele (Rick, 1945), styles remain attached to the ovary, resulting in strongly beaked fruits. Persistent style is situated on chromosome 2 and is tightly linked to green stripe (*gs*) in repulsion phase (Rick, 1966).

Three breeding lines were obtained with a pointed blossom-end morphology in the young fruits and without the associated leaf curl from breeding programs at the University of Florida and North Carolina State University. The purpose of this study was to characterize the blossom-end morphology genes in these new sources in comparison to the previously described blossom-end morphology genes and to assess the usefulness of these genes in breeding for smooth blossom-end scars.

Materials and Methods

Genetic Material

Accessions with the four previously described blossom-end morphology genes were obtained from the Tomato Genetic Stock Center at Davis, California (Table 6-1). LA 2-5 had strongly beaked, ovate fruits and the *d* (dwarf) and *s* (compound inflorescence) alleles. Fruits of LA 986 were pear shaped and the blossom-end morphology was similar to LA 2-5. LA 986 also had the alleles *d* (dwarf), *p* (peach), *s* (compound inflorescence), *r* (yellow flesh), and segregated for colorless epidermis (*y*) resulting in orange and yellow fruits. Thus, LA 986 was similar to the *bk*-source (T1079) reported by Young and MacArthur (1947). Both LA

Table 6-1. Reported blossom-end morphology gene and leaf curl rating averaged over three seasons for the genotypes used in this experiment.

Genotype	Source	Blossom-end morphology gene	Average leaf curl rating[z]
LA 2-5	TGSC[y]	*pst*	1.0
LA 986	TGSC	*bk*	1.0
LA 1787	TGSC	*Bk*-2	1.7
LA 2353	TGSC	*n*	1.4
Valerie	NK[x]	?	4.5
NC 140	NCSU[w]	?	1.2
Fla 890559-24	UF[v]	?	1.1
Fla 894413-1	UF	?	2.3

[z] Rated on a scale from 1 to 5 where 1 = no leaf curl and 5 = severe leaf curl.
[y] Tomato Genetic Stock Center in California.
[x] Northrup King seed company.
[w] North Carolina State University.
[v] University of Florida.

986 and LA 2-5 had low fruit set. Nippling due to *Bk*-2 in LA 1787 was clearly distinguishable in young fruits, but only moderately in mature fruits. LA 1787 also had *sp* (self pruning), *en* (ensiform; sword shaped sepals), and ovate fruit shape. LA 2353 segregated ovate and round fruit shape, both with strongly pointed blossom-ends. In the fall of 1989 and 1990 slight curling of the leaf margins was observed in LA 2353, similar to the *n*-source (T667V), described by Young and MacArthur (1947). However, leaf curl in LA 2353 was negligible in comparison to the leaf curl in 'Valerie' under Florida field conditions (Table 6-1). The seed from plants with ovate fruit shape bred true and was selected for this experiment, because of more prominent nippling. LA 2353 had pink fruit, as a result of colorless epidermis (*y*) with red fruit flesh. LA 2370 was also obtained because it was reported to have the *n* allele (anonymous, 1990); however, when grown in three different seasons, no nippling of significance was observed. All the accessions had two locules per fruit.

The hybrid cultivar Valerie had pointed blossom-ends as well as leaf curl and was assumed to be homozygous for both characters. The leaf curl was characterized by adaxially curled leaf margins, frequently touching each other, and adaxially protruding midribs of the leaflets (Figure 6-1). In this experiment 'Valerie' was used to identify the pointed blossom-end character associated to leaf curl widely used by commercial breeders. The breeding lines NC 140, Fla 890559-24, and Fla 894413-1 had a pointed blossom-end morphology but no significant leaf curl (Table 6-1). The pointed blossom-end character was expressed most weakly in NC 140, where it was confined to the young fruits only. The

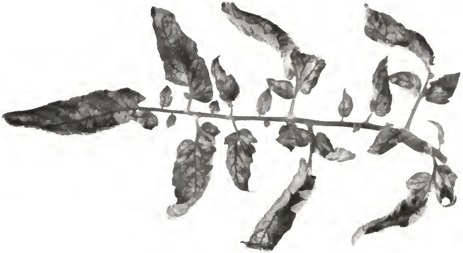

Figure 6-1. Genetic leaf curl in 'Valerie'.

character was more pronounced in Fla 890559-24 and Fla 894413-1, where some mature fruits had a pointed blossom-end. The three breeding lines and 'Valerie' had a round fruit shape. 'Valerie' and Fla 894413-1 had *sp*, whereas Fla 890559-24 segregated for *sp*, and NC 140 was sp^{+}.

The inheritance of blossom-end morphology genes in each of the genotypes in Table 6-1 was investigated in F1 and F2 generations from crosses with wildtypes. The wildtypes used were Fla 884068-1, 'Florida MH-1', and 'Horizon', all round fruited with only sporadic occurrence of pointed blossom-ends in immature fruits. All wildtypes had *sp* and 'Florida MH-1' and 'Horizon' had the jointless (*j*-2) allele. The genotypes in Table 6-1 were intercrossed and F1 and F2 generations were tested for allelism.

Experimental Procedure

Parental, F1 and F2 generations were grown at Bradenton, Florida, in three seasons, spring and fall 1990, and spring 1991, but not every cross in each season. The plants were grown in single 10-plant plots for parental and F1 generations and in single 30-plant plots for F2 generations. The plants were staked, tied, and watered by seepage irrigation. Fertilizers and pesticides were applied following recommended cultural practices. Indeterminate plants were topped at approximately 1.5 m.

The expression of blossom-end morphology was variable among fruits within a plant. Prominence of the pointed blossom-end morphology depended in part on the age of the fruit; fruits that had a pointed blossom-end when young frequently did not show any sign of protruding blossom-ends at maturity. Thus, when assessing the percentage of fruits with pointed blossom-ends, the age of the fruits needed to be taken into account. Another difficulty was the difference in fruit shape between some of the genotypes. Subjective observations suggested that nippling was more prominent on ovate and pear shaped fruits than on round or oblate fruits. A scoring technique was developed taking these considerations into account.

Per individual plant, ten young fruits (diameter 1-4 cm) and ten mature fruits were rated for presence or absence of a pointed blossom-end. The percentage pointed fruits was calculated per plant and averaged over ten plants for parental and F1 generations. To study F2 segregation in wildtype crosses, the minimum percentage of pointed fruit per plant in the mutant parent was taken as threshold value; plants with a lower percentage pointed fruits were counted as wildtype and with a higher percentage as mutant. In F2 generation allelism tests, a similar system was developed. To aid in classification, the type of blossom-end as well as fruit shape was scored per plant. The prominent, pointed, columnar blossom-end characteristic for LA 986 and LA 2-5 was scored as p-type, whereas a more rounded, less prominent blossom-end like in LA 2353 was scored as a u-type (Figure 6-2). A leaf curl rating

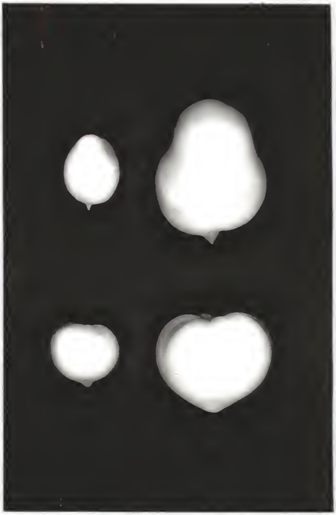

Figure 6-2. P-type blossom-end morphology in elongated fruit shape (top, LA 986) and u-type blossom-end morphology in round fruit shape (bottom, Fla 890559-24).

was given from each side of the plant on a scale from 1 to 5 (1 = no leaf curl, 5 = severe leaf curl) and averaged per plant (Table 6-1). Markers in the accessions and wildtypes were used to verify each cross.

Pistils of flowers at anthesis were observed under a binocular microscope in an attempt to identify morphology associated with the mutant genes. Observations were made on the shape and pubescence of the stylar base and on the position of the separation between style and ovary.

Results

The inheritance of previously described genes was studied to confirm previous reports, predict F2 segregation with undescribed genes, and to investigate the usefulness of these genes for blossom-end scar improvement. The pointed blossom-end morphology was generally expressed more prominently in the fall of 1990 as compared to the spring seasons of 1990 and 1991 (Table 6-2). The *pst* gene in heterozygous condition did not have the p-type blossom-end, characteristic for the homozygous recessive condition (Table 6-2). Some incomplete dominance was indicated by low percentages of u-type blossom-ends in the F1's of wildtype x LA 2-5. The F2 segregation of 'Florida MH-1' x LA 2-5 did not deviate significantly from a 3 normal to 1 mutant ratio in two seasons; however, a digenic model could not be rejected (Table 6-2). When data were pooled over seasons, a digenic model was accepted over a single gene model. Low recovery of mutants may be related to the low fertility of LA 2-5.

Table 6-2. Inheritance of described blossom-end morphology genes in F1 and F2 generations of crosses with wildtypes.

Cross[y]	Season[x]	Avg. pointed fruits (%)[z]			F2 segregation					
		P1	P2	F1	Threshold[w]	+:m	$X^2_{(3:1)}$	*P* val	$X^2_{(15:1)}$	*P* val
Fla MH-1 x LA 2-5 (*pst*)	S90	3	100(p)[v]	21(u)	100(p)	26: 3	3.32[u]	0.068	0.83[u]	0.362
Fla MH-1 x LA 2-5 (*pst*)	F90	20	100(p)	17(u)	100(p)	26: 4	2.18[u]	0.140	2.57[u]	0.109
Fla 884068-1 x LA 2-5 (*pst*)	S91	2	100(p)	17(u)	-	-	-	-	-	-
Horizon x LA 2-5 (*pst*)	S91	0	100(p)	17(u)	-	-	-	-	-	-
Fla MH-1 x LA 986 (*bk*)	S90	3	99(p)	84(u)	92(p)	23: 6	0.29[t]	0.590	14.94[t]	0.001
Fla MH-1 x LA 986 (*bk*)	F90	20	100(p)	74(u)	100(p)	25: 4	1.94[t]	0.164	2.82[t]	0.093
Fla 884068-1 x LA986 (*bk*)	S91	2	98(p)	62(u)	-	-	-	-	-	-
Fla MH-1 x LA 1787 (*Bk*-2)	S90	3	63	13	50	19: 8	0.31	0.578	25.19	<0.001
Fla MH-1 x LA 1787 (*Bk*-2)	F90	20	70	39	55	21: 9	0.40	0.527	28.88	<0.001
Fla 884068-1 x LA 1787 (*Bk*-2)	S90	4	63	19	50	20: 9	0.56	0.454	30.40	<0.001
Fla 884068-1 x LA 1787 (*Bk*-2)	F90	15	70	11	55	24: 6	0.40	0.527	9.68	0.002
Horizon x LA 1787 (*Bk*-2)	S91	0	58	24	-	-	-	-	-	-
Fla MH-1 x LA 2353 (*n*)	S90	3	88	6	67	19: 8	0.31	0.578	25.19	<0.001
Fla MH-1 x LA 2353 (*n*)	F90	20	77	21	65	20:10	1.11	0.292	37.56	<0.001
Fla 884068-1 x LA 2353 (*n*)	S90	4	88	6	67	22: 6	0.19	0.663	11.01	0.001
Horizon x LA 2353 (*n*)	S91	0	93	19	-	-	-	-	-	-

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[z] Based on ten plants and 20 fruits per plant, ten young fruits (diameter 1-4 cm) and ten mature fruits.

[y] The notation does not indicate the actual direction of the cross; wildtypes are mentioned first for clarity.

[x] Spring 1990 (S90), fall 1990 (F90), and spring 1991 (S91).

[w] Minimum percentage pointed fruit per plant in mutant parent. Used as threshold value to separate F2 individuals into wildtypes (+) and mutants (m). A p indicates that only plants with p-type blossom-ends were rated as mutant.

[v] The type of blossom-end morphology is indicated when parents differ.

[u] For data pooled over seasons, $X^2_{(3:1)} = 5.43$ ($P = 0.020$) and $X^2_{(15:1)} = 3.17$ ($P = 0.075$).

[t] For data pooled over seasons, $X^2_{(3:1)} = 1.86$ ($P = 0.173$) and $X^2_{(15:1)} = 11.96$ ($P < 0.001$).

The p-type blossom-end morphology, characteristic of the *bk* allele, was absent in the wildtype x LA 986 F1's, although 62% to 84% of the F1 fruit had u-type blossom-ends (Table 6-2). Thus, *bk* was not completely recessive with a greater percentage u-type blossom-ends in the F1 than LA 2-5 hybrids. In the F2 from 'Florida MH-1' x LA 986, only F2 plants with a p-type blossom-end were classified as homozygous recessive and deviation from a 3 normal to 1 mutant segregation was insignificant. Pooling of data over seasons resulted in rejection of a digenic model (Table 6-2). Young and MacArthur (1947) reported single recessive gene action for *bk*.

Bouwkamp and Honma (1970) reported dominant gene action for the *Bk*-2 gene, but in the present experiment *Bk*-2 inherited recessively (Table 6-2). Some pointed blossom-ends were observed in the wildtype x LA 1787 F1's, but mainly in the young fruits. Furthermore, segregation in the F2's of 'Florida MH-1' x LA 1787 and Fla 884068-1 x LA 1787 over each of two seasons fit a 3 normal to 1 mutant ratio, indicating single recessive gene action (Table 6-2). Henceforth this gene will be symbolized as *bk*-2.

The nipple tip gene (*n*) was almost completely recessive in the F1 and the F2 clearly segregated 3 normal to 1 nipple tip. Thus, the nipple tip trait in LA 2353 was regulated by a single recessive gene, confirming the results of Young and MacArthur (1947), but not the digenic model of Tikoo and Anand (1984). Absence of significant leaf curl and almost completely recessive gene action makes the *n*-gene a possibility in hybrid breeding for smooth blossom-end scars.

The inheritance of blossom-end morphology in the three breeding lines was investigated in the F1 and F2 generations as they became available (Table 6-3). The expression of pointed blossom-ends was weak in NC 140 and under some conditions it was hard to separate wildtype from mutant plants in F2 generations. Average values for the F1's of wildtype x NC 140 were low and seemed to indicate recessive gene action, although the average percentage pointed fruits for Fla 884068-1 x NC 140 tended towards dominant gene action. In the F2, however, the segregation ratio was clearly 3 normal to 1 mutant, indicating single recessive gene action (Table 6-3). These results agreed with observations on the F2 of a cross between NC 902-1, derived from NC 140, and NC 50-7, a wildtype, grown in Fletcher, North Carolina. The segregation was 30 wildtype to 17 mutant with $X^2_{(3:1)} = 3.13$ ($P = 0.077$) and $X^2_{(15:1)} = 71.81$ ($P < 0.001$). Thus the single gene model was accepted and the digenitc model clearly rejected.

Presence of u-type blossom-ends in the F1's of wildtype x Fla 890559-24 indicated incomplete dominance of the wildtype, and the degree of dominance varied with the cross and the season (Table 6-3). In the spring of 1990, the F2 segregation did not deviate from 3 normal to 1 mutant and a digenic model was rejected. However, in the fall of 1990, the F2 segregation deviated significantly from 3 normal to 1 mutant due to overrepresentation of mutants. A low threshold value, combined with incomplete dominance may have resulted in grouping of some heterozygous genotypes in the mutant class. When data were pooled over seasons, a single gene model was accepted over a digenic model (Table 6-3).

Table 6-3. Inheritance of blossom-end morphology in F1 and F2 generations of three breeding lines crossed with wildtypes.

Cross[y]	Season[x]	Avg. pointed fruits (%)[z]			F2 segregation					
		P1	P2	F1	Threshold[w]	+:m	$X^2_{(3:1)}$	*P* val	$X^2_{(15:1)}$	*P* val
Fla MH-1 x NC 140	S91	1	24	3	-	-	-	-	-	-
Fla 884068-1 x NC 140	S91	2	24	19	-	-	-	-	-	-
Horizon x NC 140	F90/S91[v]	6	20	11	17	24: 6	0.40	0.527	9.68	0.002
Fla MH-1 x Fla 890559-24	S90	3	52	30	40	23: 7	0.04[u]	0.842	14.94[u]	<0.001
Fla MH-1 x Fla 890559-24	F90	20	46	10	35	17:12	4.15[u]	0.042	61.08[u]	<0.001
Fla 884068-1 x Fla 890559-24	S91	2	50	29	-	-	-	-	-	-
Horizon x Fla 890559-24	S91	0	50	3	-	-	-	-	-	-
Fla MH-1 x Fla 894413-1	S91	1	49	12	-	-	-	-	-	-
Fla 884068-1 x Fla 894413-1	S91	2	49	15	-	-	-	-	-	-
Horizon x Fla 894413-1	S91	0	49	1	-	-	-	-	-	-
Fla 7322 x Fla 894413-1	F90/S91	35	60	35	40	23: 7	0.04	0.842	14.94	<0.001

[z] Based on ten plants and 20 fruits per plant, ten young fruits (diameter 1-4 cm) and ten mature fruits.

[y] The notation does not indicate the actual direction of the cross; wildtypes are mentioned first for clarity.

[x] Spring 1990 (S90), fall 1990 (F90), and spring 1991 (S91).

[w] Minimum percentage pointed fruit per plant in mutant parent. Used as threshold value to separate F2 individuals into wildtypes (+) and mutants (m).

[v] The F1 generation was raised in fall 1990 and the F2 generation in spring 1991. Parents were raised in both seasons.

[u] For data pooled over seasons, $X^2_{(3:1)} = 1.63$ ($P = 0.202$) and $X^2_{(15:1)} = 67.82$ ($P < 0.001$).

Fla 894413-1 was similar to Fla 890559-24 in expression of pointed blossom-ends. The F1's of wildtype x Fla 894413-1 crosses indicated primarily recessive gene action and the F2 segregation indicated acceptance of a single gene model over a digenic model (Table 6-3). In general, the expression of pointed blossom-end morphology in heterozygous condition was variable among seasons and crosses for each breeding line.

F1 and F2 generations of crosses between accessions were grown to test for allelism between the described blossom-end morphology genes. A first indication of allelism was equal average percentage of pointed fruits for parental and F1 generations. A second test for allelism involved examination of the F2 generation. The minimum percentage pointed fruits in the least pointed parent was used as a minimum threshold. Presence of F2 individuals that had a lower percentage pointed fruits than the low threshold was a strong indication of complementation, meaning control by two different blossom-end morphology genes. A high threshold was also established, based on the parent plant with the greatest pointed fruit percentage. Presence of F2 individuals with a higher expression of pointed blossom-ends than the high threshold probably indicated two different genes with additive effect in the duplicate recessive condition.

The parental and F1 generations of the cross between LA 986 (*bk*) and LA 2-5 (*pst*) were characterized by 100% p-type blossom-ends (Table 6-4). Furthermore, all plants in the F2 had 100% p-type blossom-ends, indicating that LA 986 and LA 2-5 had the same blossom-end morphology gene (Table 6-4). The F2 of LA 986 x LA 2-5 was segregating for fruit

Table 6-4. Allelism tests for described blossom-end morphology genes.

Cross[y]	Season[x]	Avg. pointed fruits (%)[z]			F2 segregation				
					Threshold[w]		Number of plants		
		P1	P2	F1	Low	High	≤ Low	Mid	≥ High
LA 986 (*bk*) x LA 2-5 (*pst*)	F90	100(p)[v]	100(p)	100(p)	100	100(p)	0	0	25
LA 1787 (*bk*-2) x LA 986 (*bk*)	S90	63(u)	99(p)	52(u)	-	-	-	-	-
LA 1787 (*bk*-2) x LA 986 (*bk*)	F90/S91[u]	70(u)	100(p)	79(u)	55	100(p)	7	20	2
LA 1787 (*bk*-2) x LA 2-5 (*pst*)	S90	63(u)	100(p)	59(u)	50	100(p)	15	13	1
LA 1787 (*bk*-2) x LA 2-5 (*pst*)	F90	70(u)	100(p)	48(u)	55	100(p)	17	6	3
LA 2353 (*n*) x LA 986 (*bk*)	S90	88(u)	99(p)	98(u)	67	100(p)	6	14	9
LA 2353 (*n*) x LA 986 (*bk*)	F90	77(u)	100(p)	78(u)	65	100(p)	10	36	11
LA 2353 (*n*) x LA 2-5 (*pst*)	S90	88(u)	100(p)	72(u)	67	100(p)	2	21	5
LA 2353 (*n*) x LA 2-5 (*pst*)	F90	77(u)	100(p)	74(u)	65	100(p)	11	16	2
LA 2353 (*n*) x LA 1787 (*bk*-2)	S90	88(u)	63(u)	49(u)	50	100	9	17	3
LA 2353 (*n*) x LA 1787 (*bk*-2)	F90	77(u)	70(u)	42(u)	55	90	10	12	6

[z] Based on ten plants and 20 fruits per plant, ten young fruits (diameter 1-4 cm) and ten mature fruits.

[y] The notation does not indicate the actual direction of the cross; wildtypes are mentioned first for clarity.

[x] Spring 1990 (S90), fall 1990 (F90), and spring 1991 (S91).

[w] Minimum and maximum percentage pointed fruit per plant in parents are low and high threshold value, respectively. These threshold values are used to separate F2 individuals into wildtypes (low), intermediate (Mid) and mutant (High) phenotypes. A p indicates that only plants with p-type blossom-ends were rated in the '≥ High' class under 'Number of plants'.

[v] The type of blossom-end morphology is indicated when parents differ.

[u] The F1 generation was raised in fall 1990 and the F2 generation in spring 1991. Parents were raised in both seasons.

shape and *p* (peach), confirming the identity of the cross. The blossom-end morphology of LA 986 and LA 2-5 was similar to that pictured for the *bk* allele in Young and MacArthur (1947; also Figure 6-1 top). Several characteristics of LA 2-5, such as *d* (dwarf) and *s* (compound inflorescence) did not match the description of the original *pst* source (E.A. Kerr, personal communication; Rick, 1945). Thus, it was assumed that LA 2-5 had the *bk* allele instead of the *pst* allele. Differences in the percentage of fruits with u-type blossom ends between wildtype x LA 986 and wildtype x LA 2-5 F1's in Table 6-2 were probably due to differences in genetic background and were possibly associated with a more ovate fruit shape of the wildtype x LA 986 F1's as compared to wildtype x LA 2-5 F1's. The single gene model concluded from F2 segregation in wildtype x LA 986 was probably appropriate, and the low recovery of mutants in the F2 of wildtype x LA 2-5 was probably related to low fertility and vigor in LA 2-5.

The F1 generation fruits of LA 1787 x LA 986 and LA 1787 x LA 2-5 averaged 48% to 79% u-type blossom-ends (Table 6-4). This could be due to either incomplete dominance of *bk* heterozygotes or multiple allelism with dominance of *bk*-2 over *bk*. In the F2 generations, several plants had a lower percentage pointed fruits than the minimum value for LA 1787 (Table 6-4), which could only be explained by the presence of two different complementing genes. Thus, the u-type blossom-ends in the F1's of LA 1787 x LA 986 and LA 1787 x LA 2-5 (Table 6-4) can be explained by incomplete dominance of heterozygous *bk*. The higher percentage of fruits with u-type blossom-ends in the F1 of LA 1787 x LA 2-5 (Table 6-4) as compared to wildtype x LA 2-5 F1's (Table 6-2) may be related to the fact that fruits in the F1 of LA 1787 x LA 2-5 were ovate, whereas in the wildtype x LA 2-5 F1's the fruits were round. The observed frequency of wildtype phenotypes in the F2 of LA 1787 x LA 986 was lower than the 9/16 expected with two recessive genes (Table 6-4), due to incomplete dominance of *bk* heterozygotes. Low plant numbers in the F2 and varying degrees of incomplete dominance of the wildtypes prevented further investigation of segregation ratios in the F2's. All plants in the '≥ High' class in Table 6-4 had 100% p-type blossom-ends, identical to the high threshold, and transgression at the upper end of the scale was not possible.

The F1 generation fruits of LA 2353 x LA 986 and LA 2353 x LA 2-5 averaged 72% to 98% u-type blossom-ends (Tables 6-2 and 6-4). The presence of wildtypes in the F2 generation indicated the involvement of two different genes (Table 6-4). The average percentage of fruits with u-type blossom-ends in the F1's was comparable for LA 2353 x LA 986 and LA 2353 x LA 2-5 (Table 6-4). The F1 of LA 2353 x LA 2-5 (Table 6-4) had a higher percentage of fruits with u-type blossom-ends than the F1 of wildtype x LA 2-5 (Table 6-2). This is probably related to the fruit shape which was ovate in the F1 of LA 2353 x LA 2-5 and round in the F1's of wildtype x LA 2-5. Transgression at the upper end of the scale was not possible.

In the F1 of LA 2353 x LA 1787, the average percentage of pointed fruits was lower than in either parent; however, it was relatively high, compared to wildtype expression (Table 6-4). The F2 segregation confirmed the presence of two complementing genes since 1/3 of the plants had a wildtype phenotype. Although *n* and *bk*-2 were recessive in the F1's of wildtype x LA 2353 and wildtype x LA 1787 (Table 6-2), the F1 of LA 2353 x LA 1787 averaged 42% to 49% u-type blossom-ends (Table 6-4). This may again be related to the round fruit shape in wildtype x LA 2353 and wildtype x LA 1787 F1's, and ovate fruit shape in the F1 of LA 2353 x LA 1787. Six F2 plants were grouped in the '≥ High' class in the fall of 1990, and four had a higher percentage u-type blossom-ends than the highest parent. This was possibly due to the combined effect of *n* and *bk*-2 in the duplicate recessive genotypes. Altogether, these results indicated that the three blossom-end morphology genes *n*, *bk*, and *bk*-2 were not allelic, which was in agreement with previous linkage experiments mapping *bk* on chromosome 2 (Butler, 1952; Young and MacArthur, 1947) and *n* on chromosome 5 (MacArthur, 1934).

A similar procedure was followed to test allelism of the blossom-end morphology genes in each of the three breeding lines with the previously described genes (Table 6-5). In the crosses involving NC 140, classification was difficult, since the average percentage pointed fruits in NC 140 was close to wildtype expression. The u-type blossom-ends in the F1 of NC 140 x LA 986 were due to previously described intermediate expression of *bk* heterozygotes, since the F2 had many wildtypes, providing evidence for separate genes (Table 6-5). Although there was a profound difference in the average percentage pointed fruits between NC 140 and LA 1787, only one plant was rated as wildtype in the F2 of NC 140 x LA 1787 (Table 6-5), which might indicate that the blossom-end morphology genes in these genotypes are allelic. However, closer examination of the segregation in the F2 population indicated that eleven plants were just above the low threshold. The low

Table 6-5. Allelism of breeding lines with described blossom-end morphology genes and each other.

Cross[y]	Season[x]	Avg. pointed fruits (%)[z]			F2 segregation				
					Threshold[w]		Number of plants		
		P1	P2	F1	Low	High	≤ Low	Mid	≥ High
NC 140 x LA 2-5 (*bk*)	F90	20(u)[u]	100(p)	14(u)	17	100(p)	15	8	1
NC 140 x LA 986 (*bk*)	F90	20(u)	100(p)	57(u)	17	100(p)	2	22	2
NC 140 x LA 1787 (*bk*-2)	F90	20	70	41	17	80	1	29	0
NC 140 x LA 2353 (*n*)	F90/S91[v]	20	77	12	17	100	11	17	0
Fla 890559-24 x LA 2-5 (*bk*)	S91	50(u)	100(p)	57(u)	-	-	-	-	-
Fla 890559-24 x LA 986 (*bk*)	F90	47(u)	100(p)	83(u)	35	100(p)	6	16	5
Fla 890559-24 x LA 1787 (*bk*-2)	S90	52	63	63	40	73	11	9	7(3p)
Fla 890559-24 x LA 1787 (*bk*-2)	F90	47	70	51	35	80	11	14	5(3p)
Fla 890559-24 x LA 2353 (*n*)	S90	52	88	31	40	100	8	19	2(1p)
Fla 890559-24 x LA 2353 (*n*)	F90	47	77	34	35	90	12	11	6(3p)
Fla 894413-1 x LA 2-5 (*bk*)	S91	49(u)	100(p)	40(u)	-	-	-	-	-
Fla 894413-1 x LA 986 (*bk*)	S91	49(u)	98(p)	74(u)	-	-	-	-	-
Fla 894413-1 x LA 1787 (*bk*-2)	F90/S91	60	70	46	40	65	17	5	8(2p)
Fla 894413-1 x LA 2353 (*n*)	F90/S91	60	77	46	40	100	17	11	2
NC 140 x Fla 890559-24	S91	24	50	18	-	-	-	-	-
NC 140 x Fla 894413-1	F90/S91	20	60	27	17	65	19	8	1
Fla 890559-24 x Fla 894413-1	F90/S91	47	60	53	35	70	14	11	5

[z] Based on ten plants and 20 fruits per plant, ten young fruits (diameter 1-4 cm) and ten mature fruits.

[y] The notation does not indicate the actual direction of the cross; wildtypes are mentioned first for clarity.

[x] Spring 1990 (S90), fall 1990 (F90), and spring 1991 (S91).

[w] Minimum and maximum percentage pointed fruit per plant in parents are low and high threshold value, respectively. These threshold values are used to separate F2 individuals into wildtypes (low), intermediate (Mid) and mutant (High) phenotypes. A p indicates that only plants with p-type blossom-ends were rated in the '≥ High' class under 'Number of plants'.

[v] The F1 generation was raised in fall 1990 and the F2 generation in spring 1991. Parents were raised in both seasons.

[u] The type of blossom-end morphology is indicated when parents differ.

threshold for LA 1787 was 55%, and in the F2 generation 21 plants had less and nine plants more than 55% pointed fruits. Thus, segregation in the F2 of NC 140 x LA 1787 was similar to the segregation expected in the F2 of LA 1787 crossed with a wildtype. The average percentage pointed fruits in the F1 of NC 140 x LA 1787 was similar to that for the F1 of 'Florida MH-1' x LA 1787 in the fall of 1990 (Table 6-2). This heterozygous expression of pointed fruits probably contributed to the low incidence of wildtypes in the F2 of NC 140 x LA 1787. The average percentage pointed fruits in the F1 of NC 140 x LA 2353 was very low and eleven wildtypes segregated in the F2, in spite of a low threshold value of only 17% (Table 6-5). The parental, F1, and F2 data together suggest that the blossom-end morphology gene in NC 140 is not identical to any of the described genes. Transgression over the high threshold value was not demonstrated and apparently the NC 140 blossom-end morphology gene did not add to the effect of the other genes in duplicate recessive condition (Table 6-5).

Wildtypes segregated in the F2 of Fla 890559-24 x LA 986, suggesting the presence of two separate genes. The high average percentage pointed fruits in the F1's of Fla 890559-24 x LA 986 and Fla 890559-24 x LA 2-5 (Table 6-5) could be explained by incomplete dominance of heterozygous *bk*. Transgression at the upper end of the scale was not possible. The parental and F1 values for average percentage of pointed fruits were similar for the cross Fla 890559-24 x LA 1787 (Table 6-5). However, wildtypes segregated in the F2 indicating the presence of two different genes. The high percentage pointed fruits in the F1 of Fla 890559-24 x LA 1787 was probably due to combination of the effects of *bk*-2 and the Fla 890559-24 blossom-end morphology gene in heterozygous condition. Six plants had a p-type blossom-end in the two F2's of Fla 890559-24 x LA 1787 combined (Table 6-5) and all of these had an ovate fruit shape. Thus the transgression at the upper end of the scale may be due to recombination of blossom-end morphology and fruit shape genes. Sufficient wildtypes segregated in the F2 of Fla 890559-24 x LA 2353 to conclude that the blossom-end morphology gene in Fla 890559-24 was not the same as *n* in LA 2353. The average percentage pointed fruits in the F1 of Fla 890559-24 x LA 2353 was close to that in the F1 of two wildtype x Fla 890559-24 crosses in Table 6-3. Transgression over the high threshold value in the F2 of Fla 890559-24 x LA 2353 was remarkable and four plants with p-type blossom-ends were observed in the two F2's combined (Table 6-5), two of which had ovate fruit shape.

The difference in phenotype of parental and F1 generations in Fla 894413-1 x LA 986 and Fla 894413-1 x LA 2-5 suggested that two different genes were involved (Table 6-5). The F2 generation was not available to confirm this. In the F2 of Fla 894413-1 x LA 1787, wildtypes were recovered suggesting the presence of two different genes. The high percentage of pointed fruits in the F1 of Fla 894413-1 x LA 1787 was probably due to combination of the effects of *bk*-2 and the Fla 894413-1 blossom-end morphology genes in heterozyous condition. Two plants in the F2 of Fla 894413-1 x LA 1787 had a p-type blossom-end and both had an ovate fruit shape. The recovery of wildtypes in the F2 of Fla 894413-1 x LA 2353 indicated that the blossom-end morphology gene in Fla 894413-1 was not the same as *n* in LA 2353. This conclusion was supported by the F1 of Fla 894413-1 x LA 2353 which had less pointed blossom-ends than either parent. However, the average percentage pointed fruits in the F1 was higher than expected based on observations in F1's of wildtype x LA 2353 (Table 6-2) and wildtype x Fla 894413-1 (Table 6-3).

Allelism of the three breeding lines with each other was tested in the last three crosses of Table 6-5. Differences between the parents and the low average percentage pointed fruits in the F1 generation of NC 140 x Fla 890559-24 suggested complementation between two different genes (Table 6-5). The F2 generation to confirm this was not available however. The presence of many wildtypes in the F2 of NC 140 x Fla 894413-1 suggested the presence of two different genes. The average percentage pointed fruits in the F1 of NC 140 x Fla 894413-1 was low and may be the result of a combination of intermediate expression of heterozygous blossom-end morphology genes in NC 140 and Fla 894413-1 (Table 6-3). In the F2 of Fla 890559-24 x Fla 894413-1 wildtypes were observed and two different genes were suggested. The relatively high percentage of pointed blossom-ends in the F1 of Fla 890559-24 x Fla 894413-1 was greater than expected, based on observations in the F1's of wildtype x Fla 890559-24 and wildtype x Fla 894413-1 (Table 6-3). These data suggest that three different genetic loci regulate the pointed blossom-end morphology in the three breeding lines. Since the phenotypes of these mutants closely resemble the nipple tip (*n*) gene described by Young and MacArthur (1947), the gene symbols proposed are *n*-2, *n*-3, and *n*-4 for nipple tip in NC 140, Fla 890559-24, and Fla 894413-1, respectively, in order of discovery.

The identity of several blossom-end morphology genes has now been established and different genes may be intercrossed to obtain smooth hybrids without pointed blossom-ends. However, the F1 data frequently indicated the presence of a higher percentage pointed blossom-ends than expected (Table 6-5). Thus, it seemed worthwhile to evaluate different hybrid combinations by calculating the average percentage pointed mature fruits (Table 6-6). NC 140 had a relatively low proportion of pointed mature fruits (diagonal in Table 6-6). This tendency was also observed in hybrid combinations involving NC 140. Although LA 2353 had a high percentage of pointed fruits, hybrid combinations involving LA 2353 had relatively few pointed fruits at maturity, especially when combined with NC 140 and Fla 890559-24. In hybrids involving Fla 894413-1 and LA 1787 generally a relatively high proportion of mature fruits was pointed (Table 6-6). These data only give a preliminary indication of the interactions between different blossom-end morphology genes. For a more thorough study, the genes should be incorporated into several isogenic backgrounds and the experiment should be repeated over seasons and locations.

'Valerie' was also included in the experiment and was crossed with the wildtypes and the genotypes in Table 6-1. However, the F1's of wildtype x 'Valerie' segregated for pointed blossom-ends. Following this observation, the parent lines were investigated at the Northrup King research facility in Naples, Florida. Surprisingly, the blossom-end morphology was different between the two parents, whereas both parents had severe leaf curl. The male parent had a very pronounced u-type blossom-end and the female parent had a conical v-shaped blossom-

Table 6-6. Average percentage pointed mature (numerator) and immature (denominator) fruits in hybrid combinations between different blossom-end morphology genes in the Fall of 1990.

	(1) *n*	(2) *n*-2	(3) *n*-3	(4) *n*-4	(5) *bk*-2
1) LA2353 (*n*)	62/91	3/19	8/57	20/71	16/68
2) NC140 (*n*-2)	-	7/36	1/38[a]	4/52	14/69
3) Fla890559-24 (*n*-3)	-	-	22/70	25/80	23/77
4) Fla894413-1 (*n*-4)	-	-	-	34/86	25/71
5) LA1787 (*bk*-2)	-	-	-	-	47/93

[a] Data from Spring 1991, values for NC140 and Fla890559-24 were 3/50 and 20/80, respectively.

end, a type that was not observed in other crosses in this experiment. Two different crosses of 'Valerie' with wildtypes were investigated and the segregation did not deviate significantly from 3 wildtype to 1 'Valerie' (data not shown). This also suggested that the 'Valerie' phenotype is related to heterozygosity at two different loci. Apparently there is more than one pointed blossom-end morphology gene associated with severe leaf curl. The relationship between the blossom-end morphology genes in 'Valerie' and the ones described above could not be further investigated because the parents were not available for further experimentation. The data on leaf curl were not accurate enough for investigation of inheritance due to problems with the Florida geminivirus, physiological leaf curl, and variable expression. It would be interesting to know whether more than one leaf curl gene can be identified in 'Valerie'.

Gardner and Nash (1987) reported that plants with a pointed blossom-end morphology could be classified by the shape and pubescence of the stylar base. Characterization of morphological traits associated with the blossom-end morphology genes used in the current experiment could be helpful in classification. In an effort to identify such morphological traits, observations were made on flowers of parental and F1 generations. The possible association of observed characteristics with blossom-end morphology genes was then evaluated in segregating F2 generations. Observations on density and position of the trichomes on the style indicated quantitative inheritance and in the F2 generations no association was observed between pointed blossom-end morphology and trichome density or position for any of the mutant genes under investigation. The position of the separation between style and ovary was not helpful either in classifying mutants. The shape of the stylar base was most useful (Figure 6-3). A long tapered stylar base and a slight bend in the style were characteristic for *bk*. In the F2 generations *bk* could be easily identified by looking at the young fruit. The *n* gene of LA 2353 had a more rounded stylar base. When observing segregation in F2 generations, only ovate fruit had the appearance of LA 2353. Thus in F2 generations where nipple tip as well as fruit shape segregate, this morphological character is not very useful.

Discussion

In the first description of the nipple tip (*n*) gene, MacArthur (1934) pointed out the difficulty of working with nipple tip due to its variable expression. In the present experiment, extensive effort was put into standardizing observations by taking the age of the fruits and within plant variation into account. In spite of this careful quantification, the expression of pointed blossom-ends varied

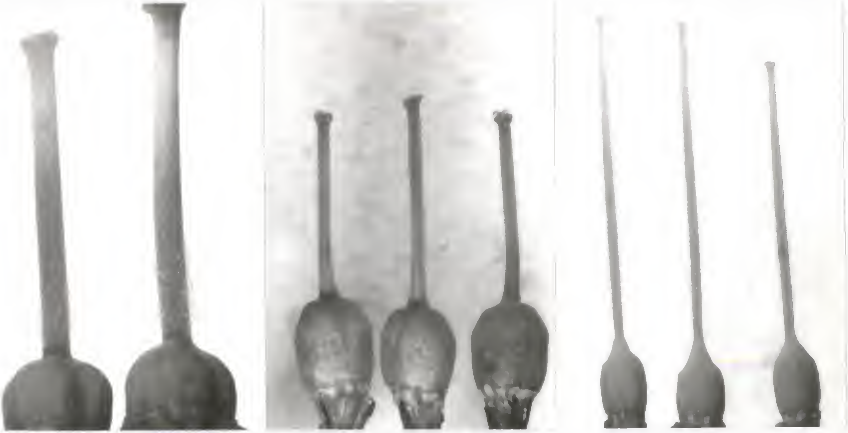

Figure 6-1. Pistil morphology at anthesis in wildtype, *bk*, and *n*, respectively from left to right

considerably within genotypes and among seasons. Frequently, hybrid expression was hard to explain, based on the expression of the parents in other crosses and could only be explained assuming varying levels of incomplete dominance. These observations indicate that expression in heterozygous and probably even in homozygous condition is dependent upon environmental conditions. The conditions promoting expression of nipple tip have not been documented. Subjective observations indicated that stress conditions tend to promote pointed blossom-ends in mature fruits. These include low light intensity and poor nutritional conditions for late fruit on a heavily fruiting plant. This variability in expression of nipple tip necessitates thorough testing of a hybrid combination for sensitivity to pointed blossom-ends before release. The genetic background probably also plays an important role in the expression of different blossom-end morphology genes. The present study suggested a promotion of expression of pointed blossom-ends in more elongated fruit shapes. However, F2 segregations indicated that not only ovate fruits had pronounced pointed blossom-ends. The difference in genetic background sometimes hindered simple interpretation of results in the present experiment. Ideally, the blossom-end morphology genes should be incorporated in several isogenic backgrounds to get a better estimate of their usefulness. However, this is a time and labor consuming process.

In spite of these problems, useful information was obtained from this experiment. An attempt was made to bring order into the terminology surrounding blossom-end morphology genes. Bouwkamp and Honma (1970) reported *bk*-2 to be dominant, however, the present experiment indicated recessive gene action, although dominance was in some crosses incomplete. The blossom-end morphology gene in LA 2-5 was assumed to be *pst*, however, in this experiment it was allelic with *bk*; LA 2-5 probably has *bk* instead of *pst*. Although the original *pst* gene described by Rick (1966) still may be available somewhere, intermediate expression in the heterozygotes was reported (Rick, 1966) and thus *pst* may not be suitable for breeding purposes anyway. The presence of at least four different nipple tip genes was established (*n*, *n*-2, *n*-3, *n*-4) and other nipple tip genes are probably being used in tomato breeding programs. One that has been used very frequently is the pointed blossom-end character associated with leaf curl, which has been referred to as nipple tip. The current data involving 'Valerie' indicated that at least two different blossom-end morphology genes are associated with leaf curl. Although no conclusions could be made on the identity of these genes, it seems unlikely that any of them is the nipple tip (*n*) gene described by MacArthur (1934), since LA 2353 did not have significant leaf curl compared to leaf curled genotypes under Florida conditions. Alternatively, the *n* gene of LA 2353 could be linked with the leaf curl locus in repulsion and a crossover occurred during early breeding line development. The leaf curl associated with pointed blossom-end morphology in much of the breeding material has been a drawback to the use of these genes in breeding smooth blossom-end scars for humid regions. Under Florida and North Carolina conditions, plants with leaf curl are more prone to be infected with foliar diseases such as bacterial spot and/or early blight. Several new sources have now been identified that do not have the leaf curl and thus are a potentially useful addition to the breeding material currently available.

In a diallel study on the inheritance of blossom-end scar size, two parents with a pointed blossom-end morphology, NC 8276 and NC 140, had remarkably smooth blossom-end scars in two very different climates (Barten et al., 1991b). This study also indicated predominantly additive inheritance of blossom-end scar size and hybrids of nippled with non-nippled parents had a scar size intermediate between the parents. Thus, to obtain hybrid fruit with smooth, pinpoint blossom-end scars, both parents need to have a pointed blossom-end. However, to prevent problems with pointed mature fruits in the hybrid, it is useful to know what blossom-end morphology genes will complement in the hybrid. In the present study several combinations involving *n*, *n*-2, and *n*-3 seemed to complement very well for nipple tip. Furthermore, since these sources of nipple tip do not have leaf curl, they could be combined with each other or types with a pointed blossom-end morphology associated with leaf curl (since leaf curl is recessive) to obtain hybrids with smooth blossom-end scars and good foliage. Hybrids between advanced lines derived from NC 140 and pointed blossom-end types with leaf curl generally have excellent smoothness without pointed mature fruits (R.G. Gardner, personal communication). Alternatively the nipple tip genes could be bred into parents to make hybrids with non-nippled but reasonably smooth parents with other desirable characteristics.

CHAPTER 7
SUMMARY AND SUGGESTIONS FOR FURTHER RESEARCH

At the start of this project, the only information available on environmental factors influencing rough blossom-end scarring (RBS) was that long term low temperatures (≥ 5 weeks) induced RBS, but also more severe fruit malformations. Experimentation with short term low temperature induction of RBS indicated that five days at 10C or six days at 18C/10C induced RBS in mature fruits of susceptible genotypes which were from 26-19 days before anthesis at the time of treatment (Chapter 2). This information proved to be helpful in relating the seemingly unpredictable occurrence of RBS under field conditions to climatological conditions during flower development. Thus, by observing weather data during early plant growth, the occurrence of RBS can be predicted and selection for smooth blossom-end scars can be more effective. Future experiments could include application of inducing low temperatures to an array of genotypes varying in sensitivity to RBS and evaluation of the potential of this induction method as a genotype screening procedure.

Identification of the developmental stage sensitive to low temperature induction of RBS facilitates investigation of the influence of other environmental factors on RBS induction. Subjective observations indicated that high wind speeds may have an RBS inducing effect. However, due to lack of knowledge on the sensitive stage during flower development, experimentation in this area failed to produce consistent results. With the information now available these experiments can be conducted more efficiently. Histological studies of induced and non-induced ovaries during and after sensitive developmental stages will help understand the cause of RBS. Such research would require electron microscopy, since RBS induction is initiated during early stages of flower differentiation. Further research into physiological backgrounds of RBS could include treatment of sensitive flowers with growth regulating substances and also measurement of endogenous concentration of such compounds after RBS induction.

Gibberellic acid (GA_3) foliar spray during early seedling growth induced fruit malformations similar to long term low temperature treatments (Sawhney, 1983) and 4-chlorophenoxyacetic acid (4-CPA) foliar spray reduced the occurrence of low temperature induced fruit malformations (Asahira et al., 1982). The effect of GA_3 and 4-CPA application on RBS induction was studied in a field experiment (Chapter 3). Flowers of 'Solar Set' and 'Horizon' were affected 8-4 and 3-0 days before anthesis, respectively. For 'Solar Set', GA_3 significantly increased the blossom-end scar size over the control and 4-CPA treatments, whereas for 'Horizon', GA_3 increased the scar size significantly over 4-CPA, but not over the control treatment. The results were not consistent over genotypes, and the effect of growth regulator treatments on RBS seemed unimportant relative to the effect of low temperatures on RBS. Reliable conclusions on the sensitivity of flowers 25-19 days before anthesis at the time of growth regulator application were impossible due to low fruit numbers. An obvious follow-up experiment would be to repeat the above experiment, focusing on flowers that are 25-19 days before anthesis at the time of application. Furthermore, 4-CPA application after low temperature induction treatment may lead to definite conclusions on the usefulness of 4-CPA spray in reducing RBS. In a contemporary experiment, Wien and Zhang (1991) reported that GA_3 foliar spray at transplanting induced RBS, increased locule numbers, and reduced the number of flowers on the early clusters. GA_3 applied at transplanting has a long term effect on plant growth and morphology, promoting vegetative growth at the cost of flower numbers and fruit growth. RBS induction may be part of this long term GA_3 induced syndrome and may be related to the increase in locule numbers.

For a long time researchers have speculated on the cause of RBS and suggestions included reduced seed numbers, increased locule numbers and reduced cell division activity at the stylar base. In this study several different approaches indicated that seed numbers are not an important factor in RBS induction (Chapter 3, Appendices B and C). This agrees with the fact that pre-anthesis developmental stages are sensitive to RBS induction.

Increased locule numbers have been associated with RBS. However, short term low temperatures induced RBS, but did not increase locule numbers in the same fruit (Chapter 2). Application of growth regulators did affect RBS, but not the number of locules (Chapter 3). Furthermore, in an array of 17 cultigens, varying in sensitivity to RBS induction, the average scar size per genotype was unrelated to the average number of locules (Appendix A). However, within most genotypes, the correlation between scar size and number of locules was significant (Appendix A). This would indicate that factors other than the number of locules determine the general level of susceptibility to RBS and that variation in the number of locules correlates with variation in scar size around this general level. Hypothetically, factors playing a role 25-19 days before anthesis could determine the general level of RBS and mechanical stress during fruit growth due to increased locule numbers could cause variation in RBS around this level. Thus, low temperature treatment of sensitive flowers would increase the general level of blossom-end scar size and within each treatment, variation in blossom-end scar size would be correlated with the number of locules around this general level.

Differentiating flowers of the first flower cluster are sensitive to low temperature induced increase in locule numbers at the 3 to 4-leaf stage (Saito and Ito, 1971) and increase in blossom-end scar size at the 5 to 6-leaf stage (Chapter 2). During flower differentiation the number of locules is determined prior to blossom-end scar size. RBS could then be induced following two alternative scenarios. In the first one, a cold spell at the 3 to 4-leaf stage increases the number of locules in the first cluster and indirectly induces RBS by increased mechanical stress. The second scenario would be RBS induction in the first cluster due to low temperatures at the 5 to 6-leaf stage as reported in Chapter 2. The relative importance of each process may depend on the genotype and sensitivity to low temperature induced locule proliferation. Multilocular (8-16 locules) genotypes are more sensitive to locule proliferation than genotypes with only a few (4-8) locules. Wien and Zhang (1991), using multilocular genotypes, reported that three weeks at 21C/10C day/night after transplanting increased blossom-end scar size as well as the average number of locules per fruit. This can be explained by a low temperature induced increase in locule number resulting in increased scar size or by the influence of the long term low temperature treatment on both processes independently. In future experiments, the period during flower differentiation that is most sensitive to low temperature induced locule proliferation could be identified relative to anthesis. Furthermore, the influence of a short term low temperature treatment on locule numbers and blossom-end scar size could be investigated for multilocular genotypes. Theoretically, in flowers that are just initiated at the time of treatment, an increase in number of locules would be expected in combination with increased blossom-end scar size, whereas in older flowers, that were 25-19 days before anthesis at the time of treatment, increased blossom-end scar size without increased locule numbers would be expected.

In this study evidence was accumulated suggesting that slow initial fruit growth is associated with increased RBS. Since initial fruit growth is mostly the result of cell division processes, these results support the hypothesis that RBS is caused by reduced cell division activity at the stylar base. Additional experiments to substantiate this hypothesis could include low temperature induction experiments, combined with measurement of fruit size two weeks after anthesis. Cell counts would be needed to confirm the relationship between early fruit size and cell division processes. Histological studies would also be helpful in this area. Nutritional studies would be hard to realize since flowers are sensitive during early development. RBS inducing treatments such a low temperature or GA_3 treatment may produce their effects by influencing assimilate partitioning in favor of vegetative growth over fruit development. RBS could thus be the result of a localized deficiency of growth substances necessary for cell division processes at the stylar base. Experiments could include low temperature treatments combined with treatments that promote partitioning to the fruits such as removal of young leaves or treatment with growth retardants.

The genetics of RBS were characterized in Chapter 4. Epistatic effects were insignificant and although both additive and dominance effects were significant, additive effects were more prominent. Although the incomplete dominance was generally towards smooth blossom-end scars, the direction of dominance may differ per individual gene and the usefulness in hybrid breeding is limited. The heritability estimates were 0.61 and 0.70 in Bradenton and Hazeva, respectively. In Chapters 4 and 5, evidence was accumulated, suggesting that genotype by environment interactions were not important if intra-plant stability was high. Chapter 5 indicated that breeding for intra-plant stability is difficult and that increased intra-plant stability only marginally increased the percentage marketable fruits per plant. Thus, selection for high intra-plant stability does not seem a promising approach in breeding for smooth blossom-end scars. However, selection against high intra-plant variation is needed to prevent instability over locations or unpredictable hybrid performance. If genotypes are smooth and have low intra-plant variation, they are likely to perform well over a wide range of environments.

A more promising approach to achieve smooth blossom-end scars is the use of blossom-end morphology genes (Chapter 6). Three different genes (*n*-2, *n*-3, *n*-4) were identified in breeding lines that had nippled fruits, but not the leaf curl generally associated with pointed blossom-ends. Current breeding efforts include backcrossing these genes into horticulturally more desirable backgrounds and using parents with complementing blossom-end morphology genes to obtain pinpoint blossom-end scars without pointed blossom-ends in mature hybrid fruit. The availability of resistant genotypes opens up interesting possibilities for further physiological research. These genotypes could be tested with RBS inducing low temperature treatments and, in combination with histological studies, the mechanism of resistance could be studied. The fact that the blossom-end morphology of a genotype has a pronounced influence on blossom-end scar size suggests that cell division processes at the stylar base during early flower development may play an important role in RBS induction.

APPENDIX A
THE RELATIONSHIP BETWEEN BLOSSOM-END SCAR SIZE AND NUMBER OF LOCULES

The occurrence of tomato fruit irregularities such as fasciation, split ovary, and catfacing has been attributed to an increased number of locules per fruit (Asahira et al., 1982; Kaname and Itagi, 1966). Wien and Zhang (1991) reported that induction of rough blossom-end scarring (RBS) coincided with an increase in average locule number and argued that RBS is caused by an increase in locule numbers. The purpose of this experiment was to investigate the relationship between RBS and locule number for 17 cultigens.

Eleven breeding lines and six cultivars (Table A-1) were transplanted to the field in Bradenton, Florida, on 3 March 1989 in a randomized complete block design with three replications and six plants per plot. Approximately 20 mature fruits were picked randomly from each plot on 26 May. A second harvest was impossible due to high incidence of bacterial wilt, incited by *Pseudomonas solanacearum* E.F. Smith. For individual fruits, the blossom-end scar size was measured, relative to fruit size as follows:

$$\mathrm{BSI} = \frac{\sqrt{a \times b}}{\sqrt{c \times d}} \times 100$$

with BSI = blossom-end scar index,

a = largest diameter of blossom-end scar,

b = smallest diameter of blossom-end scar,

c = largest diameter of equatorial fruit cross section,

d = smallest diameter of equatorial fruit cross section.

Fruits were cut in half and the number of locules was counted.

The correlation between BSI and number of locules was significant at $\alpha = 0.05$ for all genotypes, except Fla 884120-3 (Table A-1). For 'Suncoast', with many locules and high average BSI, this correlation was high, whereas the other genotypes in this experiment had moderate correlation coefficients (Table A-1). There was no pattern to the values for average BSI and average locule number in relation to the

Table A-1. Average blossom-end scar index (BSI), average number of locules, and Pearson correlation coefficient (r) between BSI and number of locules per cultigen.

Genotype	Fruit no.	Average BSI	Average locule no.	r	*P* value
Suncoast[z]	61	13.15	8.46	0.80	<0.001
Fla 884120-1[z]	60	9.91	5.40	0.73	<0.001
Fla 884120-2[z]	60	7.11	4.00	0.67	<0.001
Fla 884120-4[z]	60	7.72	4.78	0.64	<0.001
Fla 7132[z]	61	12.09	5.38	0.61	<0.001
Fla 884166-1[z]	62	4.08	4.66	0.60	<0.001
Fla 884117-1[z]	59	5.63	5.42	0.57	<0.001
Fla 884068-1[z]	55	6.28	8.25	0.52	<0.001
Fla 884088-1[z]	40	11.68	7.00	0.51	<0.001
Piedmont[y]	62	9.98	5.48	0.50	<0.001
Fla 884052-1[z]	60	6.58	6.13	0.45	<0.001
Walter[z]	55	9.46	5.87	0.41	0.002
Fla MH-1[z]	60	5.39	6.00	0.40	0.002
NC 8276[y]	58	5.37	6.40	0.40	0.004
Horizon[z]	61	11.67	4.31	0.36	0.005
Valerie	60	3.65	6.38	0.28	0.031
Fla 884120-3[z]	61	8.17	4.85	0.14	0.279

[z] From breeding program at University of Florida.
[y] From breeding program at North Carolina State University.

magnitude of the correlation coefficients. The Pearson correlation coefficient between average BSI and average locule number across cultigens is 0.13 and insignificant (*P* = 0.631). Apparently, factors other than the average number of locules determine the sensitivity of a genotype to RBS. Within genotypes, an increased number of locules was generally correlated with increased problems with blossom-end closure, even in genotypes with low average BSI (Table A-1). However, this experiment did not provide evidence for a causal relationship between increased locule numbers and RBS.

APPENDIX B
THE RELATIONSHIP BETWEEN BLOSSOM-END SCAR SIZE AND SEED SET

The Influence of Total Seed Number and Longitudinal Distribution of Seeds Within the Fruit on Blossom-end Scar Size

The occurrence of rough blossom-end scarring (RBS) in tomato has been related to incomplete fertilization (Jones et al., 1969; Sikes and Coffey, 1976), resulting in low seed numbers and reduced auxin activity (Varga and Bruinsma, 1976). Knavel and Mohr (1969) hypothesized that shortage of growth substances essential for cell division processes at the stylar base may be associated with occurrence of RBS. The purpose of the current experiment was to investigate the relationship between blossom-end scar size and seed set in total fruit, proximal fruit half, and distal fruit half.

The two breeding lines used in this experiment, NC 8276 and Fla 7132, were transplanted to the field in Bradenton, Florida, on 28 February 1988. NC 8276 generally has small blossom-end scars, whereas Fla 7132 is very susceptible to RBS. Between 31 May and 6 June, 240 mature fruits were harvested per genotype. For individual fruits, the blossom-end scar size was measured, relative to fruit size as follows:

$$BSI = \frac{\sqrt{a \times b}}{\sqrt{c \times d}} \times 100$$

with BSI = blossom-end scar index,

a = largest diameter of blossom-end scar,

b = smallest diameter of blossom-end scar,

c = largest diameter of equatorial fruit cross section,

d = smallest diameter of equatorial fruit cross section.

Fruits were weighed, cut in half equatorially and the number of locules was counted as well as the number of seeds in proximal and distal fruit halves. Mean values were compared by use of Student's t test if the sample variances were not significantly different, otherwise a t′ test was used (Steel and Torrie, 1980).

The significantly higher average BSI for Fla 7132 as compared to NC 8276 was not associated with more locules per fruit or less seed per gram fruit weight in Fla 7132 (Table B-1). A high percentage of seeds in the distal fruit half was not related to low BSI (Table B-1), indicating that the longitudinal distribution of seeds within the fruit is not a major RBS influencing factor.

Table B-1. Significance of the difference between mean values for several fruit characteristics for NC 8276 and Fla 7132.

	NC 8276	Fla 7132
Number of fruits	239	240
Blossom-end scar index	5.49	15.37***^{z}
Number of locules	6.94	6.04***^{y}
Number of seeds	173.4	150.9*^{z}
Number of seeds per gram fruit weight	0.961	0.887^{NS,y}
Percentage of seeds in distal half	62.53	65.38*^{y}

^{z,y} NS,*,***: Differences not significant or significant at $\alpha = 0.05$ or $\alpha = 0.001$ in t′ test (z) and Student's t test (y).

If RBS was due to low seed numbers, the correlation of BSI with total seed numbers and with the percentage of seeds in the distal fruit half was expected to be negative. To obtain correlation coefficients between BSI and total number of seed, the fruits were classified by the number of seeds they contained. The Pearson correlation coefficient between the average BSI and average seed number for each class was calculated per genotype (Table B-2). The correlation between BSI and total number of seeds per fruit was insignificant for each genotype (Table B-2). The correlation between BSI and the total number of seeds per gram fruit weight was obtained in a similar way. For the resistant NC 8276, this correlation was insignificant, but for the susceptible Fla 7132 it was significantly negative at $\alpha = 0.05$ (Table B-3). The correlation between BSI and percentage of seed in the distal half was significantly positive for Fla 7132 (Table B-4), indicating that an

Table B-2. Pearson correlation coefficients (r) between blossom-end scar index (BSI) and total seed number per fruit (SDNR), calculated from average values for BSI and SDNR for fruits, classified according to number of seeds per fruit.

Seed number per fruit	NC 8276			Fla 7132		
	Fruit no.	Avg. SDNR	Avg. BSI	Fruit no.	Avg. SDNR	Avg. BSI
1 - 50	27	30.2	5.77	20	32.0	24.21
51 - 100	43	77.8	6.09	54	76.2	17.74
101 - 150	45	127.6	5.03	58	123.3	13.71
151 - 200	42	178.0	4.32	48	173.4	13.81
201 - 250	24	219.7	5.86	31	224.1	12.39
251 - 300	26	270.0	5.51	13	270.9	13.30
301 - 350	15	322.4	7.07	10	326.8	12.39
351 - 500	17	408.2	5.71	6	375.3	17.98
		$r = 0.25$ $P = 0.542$			$r = -0.50$ $P = 0.202$	

Table B-3. Pearson correlation coefficients (r) between blossom-end scar index (BSI) and total seed number per gram fruit fresh weight (NRWT), calculated from average values for BSI and NRWT for fruits, classified according to number of seeds per gram fruit fresh weight.

Seed number per gram fruit fresh weight	NC 8276			Fla 7132		
	Fruit no.	Avg. NRWT	Avg. BSI	Fruit no.	Avg. NRWT	Avg. BSI
0.00 - 0.25	15	0.172	6.15	11	0.176	27.44
0.26 - 0.50	34	0.377	6.75	36	0.388	21.85
0.51 - 0.75	43	0.633	5.14	59	0.628	16.28
0.76 - 1.00	46	0.872	4.52	55	0.871	15.27
1.01 - 1.25	40	1.120	6.14	39	1.108	10.59
1.26 - 1.50	29	1.376	4.76	19	1.356	8.33
1.51 - 2.00	23	1.700	6.23	12	1.686	11.78
2.01 - 4.00	9	2.571	3.90	9	2.531	9.67
		$r = -0.59$ $P = 0.127$			$r = -0.80$ $P = 0.017$	

increase in proportion of seeds in the distal part, near the blossom-end scar, was correlated with increased BSI. This means that RBS is not caused by low seed numbers close to the blossom-end in the distal fruit half.

The linear correlation between BSI and total seed number per gram fruit weight for Fla 7132 was significant only at $\alpha = 0.05$ (Table B-3). Inspection of the data for average BSI and average seed number per gram fruit weight for Fla 7132 indicated that the relationship may be nonlinear and that the regression coefficient may be improved by adding a quadratic term to the model. PROC STEPWISE of SAS (Statistical Analysis System) was used to test whether addition of a quadratic term significantly improved the regression model. This was the case for Fla 7132 ($P = 0.006$), but not for NC 8276 ($P > 0.5$). For Fla 7132, an increase in total number of seeds per gram fruit weight was effective in

Table B-4. Pearson correlation coefficients (r) between blossom-end scar index (BSI) and percentage seed in distal fruit half (PCDST), calculated from average values for BSI and PCDST for fruits, classified according to percentage seed in distal fruit half.

Percentage seed in distal fruit half	NC 8276			Fla 7132		
	Fruit no.	Avg. PCDST	Avg. BSI	Fruit no.	Avg. PCDST	Avg. BSI
25.0 - 50.0	37	44.85	5.60	23	45.33	10.18
50.1 - 55.0	34	52.70	4.25	25	52.83	13.32
55.1 - 60.0	39	57.84	4.18	43	57.71	12.82
60.1 - 65.0	39	62.40	5.93	37	62.61	14.51
65.1 - 70.0	34	67.69	6.16	38	67.06	13.46
70.1 - 80.0	33	75.24	6.97	41	74.56	18.95
80.1 - 90.0	15	84.23	5.61	23	85.29	22.56
90.1 - 100.0	8	94.43	5.34	10	96.17	22.62
		$r = 0.33$ $P = 0.423$			$r = 0.96$ $P < 0.001$	

reducing BSI only when less than one seed was present per gram fruit weight (Figure B-1).

The Effect of Pollen Dilution on Blossom-end Scar Size

Previous studies with RBS susceptible Fla 7132 indicated that BSI correlated negatively with the total number of seeds per gram fruit weight only when less than one seed was present per gram fruit weight. Verkerk (1957) reported that seed set was affected by diluting pollen with an inert powder to 10% or 1% before self-pollination. In the current experiment the effect of pollen dilution on seed set, locule number, and BSI was investigated as well as the relationship between BSI and the number of seeds per gram fruit weight for the RBS susceptible cultivars Solar Set and Horizon.

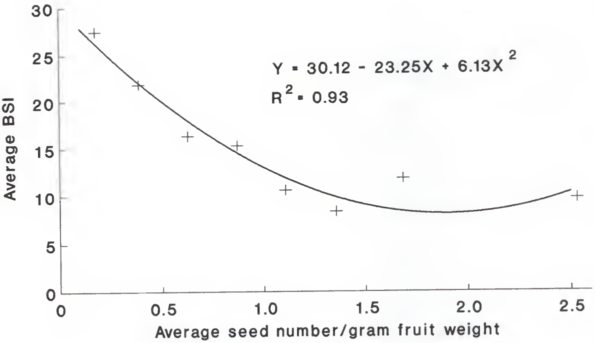

Figure B-1. The relationship between average blossom-end scar index (BSI) and the average number of seeds per gram fruit weight for Fla 7132.

Two 5-plant plots of 'Solar Set' and 'Horizon' were transplanted to the field in Bradenton on 27 February 1990. Flowers were emasculated and pollinated with a 100%, 50%, 10%, or 5% pollen mixture. Several pollen mixtures were used on a given plant. Mature fruits were harvested, weighed, and BSI was measured. The fruit was cut in half and the number of seeds and locules was counted. Due to problems with fruit set, the 5% pollen mixture treatment was eliminated from the experiment.

The data were analyzed as a 2 x 3 factorial unbalanced completely randomized design with two cultivars and three dilution treatments. LSMEANS in PROC GLM of SAS was used to calculate the least squares means for each treatment combination and the PDIFF option was used to obtain pairwise comparisons for the main effects. Multiple range tests were constructed from pairwise comparisons with α/n as the level of significance for individual pairwise comparisons, where α is the level of significance for the multiple range test and n is the number of pairwise comparisons possible.

The fruit numbers were low, especially for the 10% pollen dilution treatment (Table B-5). The cultivar x dilution treatment interaction was insignificant ($\alpha = 0.10$) for the characteristics studied (Table B-6). Pollen dilution had no significant effect on BSI and on the number of locules per fruit; however, the effect on number of seeds per fruit and number of seeds per gram fruit weight was highly significant (Table B-6). Although the average number of locules and the average number of seeds differed significantly between 'Solar Set' and 'Horizon', the average BSI was not significantly different between cultivars (Table B-7). The pollen dilution treatments induced significant differences in the number of seeds per gram fruit weight, but not in BSI (Table B-7). The average seed numbers per gram fruit weight for the different dilution treatments were below the threshold of one seed per gram fruit weight, and a negative correlation between BSI and number of seeds per gram fruit weight would be expected. However, such a relationship was not apparent for 'Solar Set' or 'Horizon' (Table B-7).

Table B-5. Number of fruits per dilution treatment.

	Solar Set	Horizon
100% pollen	9	22
50% pollen	11	11
10% pollen	5	4

Table B-6. Analysis of variance for blossom end scar index (BSI), number of locules, number of seeds per fruit, and number of seeds per gram fruit fresh weight.

Source	BSI			Locule number		
	df	MS	*P* value	df	MS	*P* value
Cultivar	1	11.030	0.235	1	12.874	<0.001
Dilution treatment	2	0.186	0.976	2	0.883	0.370
Cultivar x dilution	2	4.476	0.561	2	0.017	0.981
Error	56	7.668		56	0.873	
Total	61			61		

Table B-6. Continued

Source	Seed number			Seed number per gram		
	df	MS	*P* value	df	MS	*P* value
Cultivar	1	9,873	0.039	1	0.7415	0.032
Dilution treatment	2	23,080	<0.001	2	1.2314	<0.001
Cultivar x dilution	2	3,144	0.249	2	0.2275	0.235
Error	51	2,203		51	0.1525	
Total	56			56		

Table B-7. Average blossom-end scar index (BSI), number of locules, number of seeds per fruit, and number of seeds per gram fruit fresh weight per cultivar and dilution treatment.

	BSI	Locule no.	Seed no.	Seed no. per gram
Cultivar				
Solar Set	8.35 a[z]	5.49 a	90.0 a	0.691 a
Horizon	9.33 a	4.42 b	59.9 b	0.429 b
Dilution treatment				
100% pollen	8.89 a	4.72 a	114.5 a	0.856 a
50% pollen	8.73 a	5.09 a	77.0 b	0.537 b
10% pollen	8.89 a	5.05 a	33.4 b	0.287 b

[z] Values with the same letter are not significantly different in constructed multiple range test (see text) with $\alpha = 0.05$.

Altogether these experiments indicated that seed set does not play a major role in RBS induction and that the influence of seed set on RBS induction is genotype dependent. This agrees with previous work where induction of RBS (Rylski, 1979) and catfacing (Sikes and Coffey, 1976) was effective before anthesis, but not during or after anthesis. Apparently, factors determining the sensitivity of a genotype to RBS play a role before anthesis.

APPENDIX C
THE RELATIONSHIP BETWEEN BLOSSOM-END SCAR SIZE AND REGULARITY OF FRUIT SHAPE AND SEED DISTRIBUTION OVER LOCULES

Subjective observations indicated that the blossom-end scar of tomato has a tendency to follow fruit shape and that irregularly shaped fruits frequently have large blossom-end scars. Sikes and Coffey (1976) suggested that uneven seed distribution within the fruit may be related to rough blossom-end scarring (RBS). The purpose of this experiment was to investigate the relationship between fruit regularity and blossom-end scar size for 21 cultigens. Furthermore, the relationship between blossom-end scar size and regularity of seed distribution over locules was studied for 'Horizon'.

Thirteen advanced breeding lines and eight cultivars (Table C-1) were transplanted to the field in Bradenton, Florida, on 28 February 1988 in a randomized complete block design with three replications and five plants per plot. Twenty mature fruits were picked randomly from each plot on 31 May and on 18 June. For individual fruits, the blossom-end scar size was measured, relative to fruit size as follows:

$$\mathrm{BSI} = \frac{\sqrt{a \times b}}{\sqrt{c \times d}} \times 100$$

with BSI = blossom-end scar index,

a = largest diameter of blossom-end scar,

b = smallest diameter of blossom-end scar,

c = largest diameter of equatorial fruit cross section,

d = smallest diameter of equatorial fruit cross section.

Regularity of fruit shape was assessed visually from 1 to 10 with 1 = very irregular, fasciated fruit shape and 10 = perfectly round fruits without any protruding locules. Regularity of fruit shape was also calculated as c - d and c/d, with c and d as described before.

Thirty-six plants of 'Horizon' were transplanted to the field in Bradenton on 6 March 1988. During three harvests, on 25 May, 30 May, and 5 June, a total of 480 fruits were picked. For individual fruits, the BSI and a visual regularity score were recorded. The fruits were then cut in half and the number of seeds in each locule was counted. Per fruit, the coefficient of variation in number of seeds per locule was calculated and used as a parameter for regularity of seed distribution over locules.

If irregularity of fruit shape coincided with large blossom-end scars, the correlation coefficient between BSI and visual regularity score was expected to be negative. The Pearson correlation coefficient between BSI and visual regularity score was significantly negative at $\alpha = 0.05$ for 14 genotypes (Table C-1). There was no pattern to the values for average BSI and average visual regularity score in relation to the magnitude of the correlation coefficients. The Pearson correlation coefficient between average BSI and average visual regularity score across cultigens was -0.21 and insignificant (P = 0.351).

Table C-1. Average blossom-end scar index (BSI), average regularity of fruit shape, and Pearson correlation coefficient (r) between BSI and visual regularity score per genotype, based on 120 fruits.

Genotype	Average BSI	Average regularity[z]	r	P value
Fla 7131[y]	8.03	6.43	0.02	0.864
Valerie	4.09	6.05	-0.02	0.830
Mt. Delight[x]	7.60	7.20	-0.03	0.747
P1061[x]	4.80	6.92	-0.10	0.263
Horizon[y]	11.32	6.03	-0.11	0.238
P1060[x]	8.97	6.51	-0.13	0.144
Piedmont[x]	8.22	7.12	-0.14	0.121
Fla MH-1[y]	8.85	6.00	-0.20	0.025
NC 8276[x]	5.04	6.30	-0.23	0.012
Fla 7181[y]	10.93	6.34	-0.27	0.003
Walter[y]	9.53	6.06	-0.27	0.003
[Horizon x (NC 8276 x Suncoast)]-F6[y]	5.85	6.16	-0.27	0.003
NC 140[x]	5.37	6.70	-0.36	<0.001
(NC 8276 x Suncoast)-F4[y]	7.04	6.30	-0.36	<0.001
Summit[x]	7.29	6.58	-0.40	<0.001
[Horizon x (NC 8276 x Suncoast)]-F6[y]	6.81	6.00	-0.45	<0.001
[Horizon x (NC 8276 x Suncoast)]-F6[y]	8.27	5.98	-0.45	<0.001
Fla 7132[y]	12.80	6.20	-0.45	<0.001
Suncoast[y]	11.90	6.17	-0.49	<0.001
[Fla 7155 x (NC 8276 x Suncoast)]-F6[y]	7.55	5.08	-0.50	<0.001
(NC 8276 x Suncoast)-F4[y]	10.44	5.58	-0.57	<0.001

[z] Assessed visually from 1 to 10, with 10 for a perfectly round fruit.
[y] From breeding program at University of Florida.
[x] From breeding program at North Carolina State University.

When calculating regularity of fruit shape from largest and smallest fruit diameters, the correlation with visual regularity scores varied from -0.20 to -0.50, depending on the genotype. Correlation coefficients between BSI and calculated regularity score were positive and varied between genotypes, similar to Table C-1 (data not shown). The Pearson correlation coefficient between average BSI and average calculated regularity score across cultigens was 0.34 (P = 0.129) and 0.35 (P = 0.115), when regularity was calculated as c - d and c/d, Table C-2. Pearson correlation coefficient (r) bwteen several fruit characteristics and the coefficient of variation for seed distribution over locules, based on 480 'Horizon' fruits.

	r	*P* value
Number of locules	0.07	0.125
Blossom-end scar index	0.06	0.202
Visual regularity score	-0.16	<0.001
Fruit size[z]	-0.23	<0.001
Total number of seeds	-0.51	<0.001

[z] Average of largest and smallest diameter of equatorial fruit cross section.

respectively. Thus, the average BSI of a genotype is unrelated to average regularity of fruit shape.

The Pearson correlation coefficient between BSI and regularity of seed distribution over locules was insignificant for 'Horizon' (Table C-2). The correlation between visual regularity score and regularity of seed distribution over locules was significant, but only moderate. An increasing number of seeds per fruit correlated significantly with a more even distribution of these seeds over the locules (Table C-2). Variation in size of locules was not evaluated, but may play a role in these relationships.

Although in field situations irregular fruits repeatedly have large blossom-end scars, the correlation coefficients in Tables C-1 and C-2 indicate the absence of a strong relationship between BSI and regularity of fruit shape and seed distribution over locules. This inconsistency may be due to underrepresentation of extremes when fruits are harvested at random, resulting in lower correlation coefficients than expected. A better approach may be to harvest an equal amount of fruits in each fruit shape regularity class.

APPENDIX D
GENETIC AND ENVIRONMENTAL VARIATION IN BLOSSOM-END SCAR SIZE DURING EARLY AND LATE HARVESTS

Field observations under Florida spring conditions led to the impression that rough blossom-end scarring (RBS) in tomato generally was more prominent in fruits harvested early in the season than in those harvested late in the season. The purpose of this experiment was to investigate genetic and environmental variation in blossom-end scar size during early and late harvests.

Five-week old seedlings of thirteen advanced breeding lines and eight cultivars (Table D-1) were transplanted to the field (EauGallie fine sand) in Bradenton, Florida, on 28 February 1988 in a randomized complete block design with three replications and five plants per plot. The plants were staked, tied, and watered by seepage irrigation. Fertilizer and pesticide applications were made following recommended cultural practices. Twenty mature fruits were picked randomly from each plot on 31 May (= early harvest) and on 18 June (=late harvest). For individual fruits, the blossom-end scar size was measured, relative to fruit size as follows:

$$BSI = \frac{\sqrt{a \times b}}{\sqrt{c \times d}} \times 100$$

with BSI = blossom-end scar index,

a = largest diameter of blossom-end scar,

b = smallest diameter of blossom-end scar,

c = largest diameter of equatorial fruit cross section,

d = smallest diameter of equatorial fruit cross section.

Analysis of variance (ANOVA) and Duncan's multiple range tests were performed on the average BSI per plot for each harvest separately (Tables D-1 and D-2). For calculation of the coefficient of variation (CV) in BSI per genotype, BSI values for individual fruits were pooled over replications since ANOVA indicated insignificant replication effects for average BSI.

'Valerie' performed better than the other genotypes investigated in the early as well as the late harvest (low BSI in Tables D-1 and D-2). 'Horizon', 'Suncoast', and Fla 7132 exhibited relatively large blossom-end scars in both harvests. The correlation between the early and the late harvest for average BSI per genotype was significant ($r = 0.73$; $P < 0.001$). A t' test (Steel and Torrie, 1980) indicated that the average BSI was significantly higher in the early harvest than in the late harvest ($\alpha = 0.05$). The variance in average BSI between genotypes was also significantly higher for the early harvest as compared to the late harvest (F test; $\alpha = 0.05$). These results indicate that selection for small blossom-end scars can best be done by observing early fruits, taking advantage of the high expression of BSI and the high variation between genotypes. Selection based on early fruit justified by a good correlation between early and late performance.

Pearson correlation coefficients (r) between CV in BSI and average BSI per genotype were insignificant, $r = 0.12$ ($P = 0.615$) and $r = 0.16$

Table D-1. Average blossom-end scar index (BSI) and coefficient of variation (CV) in BSI for 21 cultigens in the early harvest, based on 60 fruits.

Genotype	Average BSI[x]	DMRT[w]	CV in BSI
Valerie	5.08	A	64.2
P1061[z]	5.37	AB	33.0
NC 8276[z]	5.78	ABC	76.7
NC 140[z]	6.70	ABCD	65.6
[Horizon x (NC 8276 x Suncoast)]-F6[y]	7.60	ABCDE	83.0
Summit[z]	8.05	ABCDE	69.7
(NC 8276 x Suncoast)-F4[y]	8.68	ABCDEF	96.5
Mt. Delight[z]	8.85	BCDEF	33.4
Piedmont[z]	9.21	CDEF	37.4
P1060[z]	9.70	DEFG	27.3
[Fla 7155 x (NC 8276 x Suncoast)]-F6[y]	10.03	DEFGH	107.4
[Horizon x (NC 8276 x Suncoast)]-F6[y]	10.16	DEFGH	104.6
Fla 7131[y]	10.43	DEFGHI	57.5
Walter[y]	10.84	EFGHI	49.9
Florida MH-1[y]	12.00	FGHI	104.6
[Horizon x (NC 8276 x Suncoast)]-F6[y]	12.03	FGHI	106.6
Fla 7181[y]	13.37	GHIJ	63.9
Suncoast[y]	13.55	HIJ	58.0
Horizon[y]	13.90	IJ	53.2
(NC 8276 x Suncoast)-F4[y]	14.06	IJ	84.4
Fla 7132[y]	16.09	J	57.1
$\overline{BSI}$:	10.07	CV_{pooled}:	76.7
s^2_{BSI}:	9.47		

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[z] From breeding program at North Carolina State University.

[y] From breeding program at University of Florida.

[x] Analysis of variance indicated significant genotype effect ($P < 0.001$).

[w] Duncan's multiple range test for average BSI; means followed by the same letter are not significantly different at $\alpha = 0.05$.

($P = 0.492$) for the early and late harvest, respectively. This means that genotypes with high average BSI did not necessarily have high CV in BSI. Especially inbred lines from the cross NC 8276 x Suncoast had a high CV in BSI in the early harvest and 'Florida MH-1' exhibited much variation in BSI in both harvests (Tables D-1 and D-2). The values for CV in BSI were significantly higher in the early harvest as compared to

Table D-2. Average blossom-end scar index (BSI) and coefficient of variation (CV) in BSI for 21 cultigens in the late harvest, based on 60 fruits.

Genotype	Average BSI[x]	DMRT[w]	CV in BSI[v]
Valerie	3.10	A	45.0
[Horizon x (NC 8276 x Suncoast)]-F6[z]	3.45	AB	35.2
NC 140[y]	4.05	ABC	37.2
[Horizon x (NC 8276 x Suncoast)]-F6[z]	4.11	ABC	60.7
P1061[y]	4.23	ABCD	33.2
NC 8276[y]	4.30	ABCD	49.0
[Horizon x (NC 8276 x Suncoast)]-F6[z]	4.52	BCDE	45.3
[Fla 7155 x (NC 8276 x Suncoast)]-F6[z]	5.07	CDE	42.5
(NC 8276 x Suncoast)-F4[z]	5.41	DEF	42.2
Fla 7131[z]	5.63	EFG	39.6
Florida MH-1[z]	5.69	EFG	63.1
Mt. Delight[y]	6.35	FGH	47.2
Summit[y]	6.53	FGH	54.8
(NC 8276 x Suncoast)-F4[z]	6.81	GH	63.0
Piedmont[y]	7.23	HI	31.9
Walter[z]	8.22	IJ	47.3
P1060[y]	8.25	IJ	41.4
Fla 7181[z]	8.49	JK	36.5
Horizon[z]	8.75	JK	42.2
Fla 7132[z]	9.52	KL	45.4
Suncoast[z]	10.24	L	61.4
$\overline{BSI}$:	6.19	CV_{pooled}:	50.4
s^2_{BSI}:	4.39		

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[z] From breeding program at University of Florida.

[y] From breeding program at North Carolina State University.

[x] Analysis of variance indicated significant genotype effect ($P < 0.001$).

[w] Duncan's multiple range test for average BSI; means followed by the same letter are not significantly different at $\alpha = 0.05$.

[v] Analysis of variance indicated insignificant genotype effect ($P = 0.055$).

the late harvest (t′ test; $\alpha = 0.05$). For each harvest, the CV in BSI was pooled over genotypes as follows:

$$CV_{pooled} = \sqrt{s^2_{pooled}}/\overline{BSI}.$$

The CV_{pooled}, an estimate for the environmental variability (Falconer, 1981), was higher for the early harvest than the late harvest (Tables

D-1 and D-2). Environmental factor(s) during early spring may cause an increased expression of RBS as well as increased variation in BSI between and within genotypes. Additionally, cultigens may be more sensitive to induction of RBS at early stages in their development than at later stages.

APPENDIX E

A THEORETICAL CONSIDERATION OF GENERATION MEANS ANALYSIS OVER TWO OR MORE ENVIRONMENTS AND TWO NEW SPREADSHEETS, ENVNOSEG AND ENVSEG, FOR ANALYSIS BY MICROCOMPUTER

Introduction

A frequently used method to obtain information on genetic regulation of a characteristic is generation means analysis, generally involving parental (P1, P2), F1, F2, and backcross (BCP1, BCP2) generations. The generation means analysis is based on the average and standard error for each population and has been described in detail by Mather and Jinks (1971). Recently a Lotus 1-2-3 spreadsheet (JNTSCALE.WK1) for microcomputers has become available to facilitate calculations (Ng, 1990).

The analysis generally starts with a joint scaling test as described by Cavalli (1952) to test the adequacy of the additive-dominance model including m, [d], and [h]. In the joint scaling test, weighted least squares estimates are obtained for genetic parameters, based on the generation means with as weight the reciprocal of the squared standard error of each mean (Cavalli, 1952). The reason for weighting is that population means are not obtained with the same precision. These estimates are then used to calculate expected generation means and the weighted sum of squared deviations of observed from expected generation means is obtained. The goodness of fit of the model can be tested by assuming that this sum of squares minimized in the fitting process has a chi-square (X^2) distribution with a number of degrees of freedom equal to the number of generation means minus the number of parameters estimated. If the joint scaling test indicates inadequacy of the additive-dominance model, epistatic effects are significant and the parameters in the full genetic model, including epistatic effects [i], [j], and [l], are estimated using the perfect fit solutions of Jinks and Jones (1958). The advantage of a joint scaling test over the individual scaling tests described by Mather (1949) is that it can be used to test adequacy of any less than full model.

Frequently, the populations for generation means analysis are grown in more than one season or location and a combined genetic analysis over two or more environments is required. The advantage of such a combined analysis is that information can be obtained on the interaction of genetic parameters with the environments. Furthermore, if the interaction is insignificant, genetic parameters can be estimated more accurately by combining data over environments. This paper presents a theoretical consideration of generation means analysis over two or more environments and describes two new Lotus 1-2-3 spreadsheets (ENVNOSEG.WK1 and ENVSEG.WK1) that can be used for generation means analysis over two environments on a microcomputer. The paper also describes a generation means analysis using SAS (Statistical Analysis System), which is especially useful for more complicated or uncommon genetic models.

Generation Means Analysis Over Two Environments

Nonsegregating Generations

If epistatic effects are assumed negligible, it is sufficient to grow P1, P2, and F1 generations in two environments to obtain estimates for m, [d], [h], and the interaction of these parameters with the environments. Following the notation of Mather and Jinks (1971), the parameter e_1 represents the deviation of the mean phenotypes in each environment from the overall mean (m). Thus, e_1 is positive in the environment that has the greater expression and negative in the other environment. The full genetic model in this case includes e_1 and the interactions of [d] and [h] with e_1, represented as Gde_1 and Ghe_1 (Table E-1). Joint scaling tests can be conducted to estimate parameters and to test adequacy of any model that is less than full. To test the significance of genotype by environment interaction, the joint scaling

Table E-1. Expected generation means in a nonepistatic model over two environments.

Generation	Environment	Parameter					
		m	[d]	[h]	e_1[z]	Gde_1	Ghe_1
P1	1	1	1	0	1	1	0
F1	1	1	0	1	1	0	1
P2	1	1	-1	0	1	-1	0
P1	2	1	1	0	-1	-1	0
F1	2	1	0	1	-1	0	-1
P2	2	1	-1	0	-1	1	0

[z] If $e_1 > 0$, environment 1 has the greater expression, if $e_1 < 0$, environment 2 has the greater expression.

test includes the parameters m, [d], [h], and e_1. The distribution of the weighted sum of squared deviations of observed from expected generation means approaches X^2 with 2 degrees of freedom. The six parameters in the full model can be estimated using perfect fit solutions and the variance of these estimates can then be used to test significance (Table E-2). Demonstrating the adequacy of a model with less than six parameters by the joint scaling test is equivalent to finding the excluded parameters to be insignificant in the full model.

ENVNOSEG.WK1 is a new Lotus 1-2-3, Rel 2.1 spreadsheet for generation means analysis of nonsegregating generations over two environments. The program takes generation means, sample sizes, and population variances (as calculated by @VAR in Lotus 1-2-3) as input for each generation in each environment (Figure E-1). The spreadsheet then calculates the sample variance by correcting population variances with n/(n - 1), where n is the sample size. ENVNOSEG.WK1 performs a joint scaling test for the additive-dominance model without genotype by environment interactions. Estimates and standard errors of parameters are provided and significance can be tested with Student's t test (Figure E-1). If the X^2 test indicates significant environmental interaction, Gde_1 and Ghe_1 are included in the model and parameters are estimated using the perfect fit solutions of Table E-2. Standard errors are calculated and t' tests supply information on the significance of parameters in the full model (Figure E-1).

The calculations in ENVNOSEG.WK1 differ from Mather and Jinks (1971) in several ways. In the joint scaling test, Mather and Jinks write the normal equations that lead to the least squares estimates of

Table E-2. Perfect fit estimates for parameters in the full nonepistatic model over two environments.

$\hat{m} = 1/4(\bar{P}1.1+\bar{P}2.1+\bar{P}1.2+\bar{P}2.2)$[z]

$[\hat{d}] = 1/4(\bar{P}1.1-\bar{P}2.1+\bar{P}1.2-\bar{P}2.2)$

$[\hat{h}] = 1/2(\bar{F}1.1+\bar{F}1.2)-1/4(\bar{P}1.1+\bar{P}2.1+\bar{P}1.2+\bar{P}2.2)$

$\hat{e}_1 = 1/4(\bar{P}1.1+\bar{P}2.1-\bar{P}1.2-\bar{P}2.2)$

$\hat{G}de_1 = 1/4(\bar{P}1.1-\bar{P}2.1-\bar{P}1.2+\bar{P}2.2)$

$\hat{G}he_1 = 1/2(\bar{F}1.1-\bar{F}1.2)-1/4(\bar{P}1.1+\bar{P}2.1-\bar{P}1.2-\bar{P}2.2)$

$Var(\hat{m}) = 1/16\{Var(\bar{P}1.1)+Var(\bar{P}2.1)+Var(\bar{P}1.2)+Var(\bar{P}2.2)\}$

$Var([\hat{d}]) = 1/16\{Var(\bar{P}1.1)+Var(\bar{P}2.1)+Var(\bar{P}1.2)+Var(\bar{P}2.2)\}$

$Var([\hat{h}]) = 1/4\{Var(\bar{F}1.1)+Var(\bar{F}1.2)\}+1/16\{Var(\bar{P}1.1)+Var(\bar{P}2.1)+Var(\bar{P}1.2)+Var(\bar{P}2.2)\}$

$Var(\hat{e}) = 1/16\{Var(\bar{P}1.1)+Var(\bar{P}2.1)+Var(\bar{P}1.2)+Var(\bar{P}2.2)\}$

$Var(\hat{G}de_1) = 1/16\{Var(\bar{P}1.1)+Var(\bar{P}2.1)+Var(\bar{P}1.2)+Var(\bar{P}2.2)\}$

$Var(\hat{G}he_1) = 1/4\{Var(\bar{F}1.1)+Var(\bar{F}1.2)\}+1/16\{Var(\bar{P}1.1)+Var(\bar{P}2.1)+Var(\bar{P}1.2)+Var(\bar{P}2.2)\}$

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[z] P1.1 indicates P1 in environment 1, P1.2 indicates P1 in environment 2, etc.

the parameters in the genetic model as follows:

$$J \cdot \hat{M} = S,$$

with J = Information matrix,

$\hat{M}$ = Vector of parameters to be estimated,

S = Vector of scores.

Although not explicitly explained by Mather and Jinks, these matrices are calculated as follows:

$$J = C' \cdot N \cdot S^{-1} \cdot C \quad \text{and}$$

$$S = C' \cdot N \cdot S^{-1} \cdot Y,$$

With ′ indicating the transpose and $^{-1}$ the inverse, and matrices Y, N, S, and C as described by Rowe and Alexander (1980):

Y = Vector of the generation means,

N = Matrix with the number of individuals on which each generation mean is based as diagonal elements,

S = Matrix with the sample variance of each generation mean as diagonal elements,

C = Matrix of expected generation means in terms of parameters in the genetic model.

The parameters in the genetic model can then be estimated as follows (Mather and Jinks, 1971):

$$\hat{M} = J^{-1} \cdot S,$$

which is equivalent to (Rowe and Alexander, 1980):

$$\hat{M} = (C' \cdot N \cdot S^{-1} \cdot C)^{-1} \cdot C' \cdot N \cdot S^{-1} \cdot Y.$$

Following Rowe and Alexander (1980), variances of parameter estimates are calculated as the product of the diagonal elements of $(C' \cdot N \cdot S^{-1} \cdot C)^{-1}$

and $X^2/(k - p)$ with $X^2 = (Y - \hat{Y})' \cdot (N \cdot S^{-1}) \cdot (Y - \hat{Y})$, k = the number of generation means, and p = the number of parameters estimated. Significance of the parameters can be tested with Student's t test with k - p degrees of freedom (Rowe and Alexander, 1980). When calculating the variances of parameter estimates, Mather and Jinks fail to multiply the diagonal elements of J^{-1} with $X^2/(k - p)$, and consequently their standard errors are incorrect by a factor of $\sqrt{X^2/(k - p)}$ (Rowe and Alexander, 1980). ENVNOSEG.WK1 calculates the standard errors of parameter estimates and performs Student's t tests according to Rowe and Alexander (1980).

When testing for significance of parameters in the full genetic model, Mather and Jinks (1971) and also Ng (1990) suggest use of Student's t test. However, the standard errors can not be assumed to be the same for all generation means, which renders Student's t test inappropriate. Instead, a t' test needs to be used as shown in Figure E-1. The "effective degrees of freedom" are calculated according to the procedure of Satterthwaite (1946).

Segregating Generations

To test for significance of epistatic effects and their interaction with environments, the parental, F1, F2, and backcross generations need to be grown in both environments. The full genetic model includes [i], [j], [l], and their interactions with e_1, which are represented by Gie_1, Gje_1, and Gle_1 (Table E-3). Trigenic and higher order interactions are assumed to be insignificant, and the interacting

Table E-3. Expected generation means in digenic interaction model over two environments.

Generation	Environment	Parameter											
		m	[d]	[h]	[i]	[j]	[l]	e_1[z]	Gde_1	Ghe_1	Gie_1	Gje_1	Gle_1
P1	1	1	1	0	1	0	0	1	1	0	1	0	0
BCP1	1	1	½	½	¼	¼	¼	1	½	½	¼	¼	¼
F1	1	1	0	1	0	0	1	1	0	1	0	0	1
F2	1	1	0	½	0	0	¼	1	0	½	0	0	¼
BCP2	1	1	-½	½	¼	-¼	¼	1	-½	½	¼	-¼	¼
P2	1	1	-1	0	1	0	0	1	-1	0	1	0	0
P1	2	1	1	0	1	0	0	-1	-1	0	-1	0	0
BCP1	2	1	½	½	¼	¼	¼	-1	-½	-½	-¼	-¼	-¼
F1	2	1	0	1	0	0	1	-1	0	-1	0	0	-1
F2	2	1	0	½	0	0	¼	-1	0	-½	0	0	-¼
BCP2	2	1	-½	½	¼	-¼	¼	-1	½	-½	-¼	¼	-¼
P2	2	1	-1	0	1	0	0	-1	1	0	-1	0	0

[z] If $e_1 > 0$, environment 1 has the greater expression, if $e_1 < 0$, environment 2 has the greater expression.

genes are assumed to be unlinked. Joint scaling tests can be conducted on any model with less then twelve parameters. To test the adequacy of the additive-dominance model, m, [d], [h], and e_1 are included in the joint scaling test. Inadequacy of the additive-dominance model usually indicates either the presence of genotype by environment interaction or significant epistatic effects. The significance of genotype by environment interactions in a nonepistatic model can be tested in a joint scaling test including parameters m, [d], [h], e_1, Gde_1, and Ghe_1. To test for significance of epistatic effects, m, [d], [h], [i], [j], [l], and e_1 are included in the joint scaling test. If the generation means data do not fit any of the above models, a genetic model including interaction of epistatic effects with the environment is needed. The perfect fit solutions (Table E-4) and their variances can be used to test significance of each parameter in the full model. If the

Table E-4. Perfect fit estimates of parameters in the full digenic interaction model over two environments.

$$\begin{aligned}
\hat{m}^{z} &= 1/4(\overline{P1.1}+\overline{P2.1}+\overline{P1.2}+\overline{P2.2})+2(\overline{F2.1}+\overline{F2.2})-\overline{BCP1.1}-\overline{BCP2.1}-\overline{BCP1.2}-\overline{BCP2.2}^{y} \\
[\hat{d}] &= 1/4(\overline{P1.1}-\overline{P2.1}+\overline{P1.2}-\overline{P2.2}) \\
[\hat{h}] &= -3/4(\overline{P1.1}+\overline{P2.1}+\overline{P1.2}+\overline{P2.2})-1/2(\overline{F1.1}+\overline{F1.2})-4(\overline{F2.1}+\overline{F2.2})+3(\overline{BCP1.1}+\overline{BCP2.1}+\overline{BCP1.2}+\overline{BCP2.2}) \\
[\hat{i}] &= \overline{BCP1.1}+\overline{BCP2.1}+\overline{BCP1.2}+\overline{BCP2.2}-2(\overline{F2.1}+\overline{F2.2}) \\
[\hat{j}] &= -1/2(\overline{P1.1}-\overline{P2.1}+\overline{P1.2}-\overline{P2.2})+\overline{BCP1.1}-\overline{BCP2.1}+\overline{BCP1.2}-\overline{BCP2.2} \\
[\hat{l}] &= 1/2(\overline{P1.1}+\overline{P2.1}+\overline{P1.2}+\overline{P2.2})+\overline{F1.1}+\overline{F1.2}+2(\overline{F2.1}+\overline{F2.2})-2(\overline{BCP1.1}+\overline{BCP2.1}+\overline{BCP1.2}+\overline{BCP2.2}) \\
\hat{e}_1 &= 1/4(\overline{P1.1}+\overline{P2.1}-\overline{P1.2}-\overline{P2.2})+2(\overline{F2.1}-\overline{F2.2})-\overline{BCP1.1}-\overline{BCP2.1}+\overline{BCP1.2}+\overline{BCP2.2} \\
\hat{G}de_1 &= 1/4(\overline{P1.1}-\overline{P2.1}-\overline{P1.2}+\overline{P2.2}) \\
\hat{G}he_1 &= -3/4(\overline{P1.1}+\overline{P2.1}-\overline{P1.2}-\overline{P2.2})-1/2(\overline{F1.1}-\overline{F1.2})-4(\overline{F2.1}-\overline{F2.2})+3(\overline{BCP1.1}+\overline{BCP2.1}-\overline{BCP1.2}-\overline{BCP2.2}) \\
\hat{G}ie_1 &= \overline{BCP1.1}+\overline{BCP2.1}-\overline{BCP1.2}-\overline{BCP2.2}-2(\overline{F2.1}-\overline{F2.2}) \\
\hat{G}je_1 &= -1/2(\overline{P1.1}-\overline{P2.1}-\overline{P1.2}+\overline{P2.2})+\overline{BCP1.1}-\overline{BCP2.1}-\overline{BCP1.2}+\overline{BCP2.2} \\
\hat{G}le_1 &= 1/2(\overline{P1.1}+\overline{P2.1}-\overline{P1.2}-\overline{P2.2})+\overline{F1.1}-\overline{F1.2}+2(\overline{F2.1}-\overline{F2.2})-2(\overline{BCP1.1}+\overline{BCP2.1}-\overline{BCP1.2}-\overline{BCP2.2})
\end{aligned}$$

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[z] The variance of each estimate is obtained by summing the variances for individual generation means and squaring their coefficient as shown in Table E-2.

[y] P1.1 indicates P1 in environment 1, P1.2 indicates P1 in environment 2, etc.

interaction between genetic parameters and the environment is significant, analysis per individual environment is needed following the general procedures of Mather and Jinks (1971). If genetic parameters do not interact significantly with environments, they are estimated more accurately when analysis is combined over environments.

The data for genetic analyses are taken on an arbitrary scale, and rescaling may be needed. Reasons for rescaling are nonnormality of nonsegregating populations or dependence of nonheritable variation on genotype. The nonsegregating populations can be used to check for nonheritable variation that is genotype dependent by testing for homogeneity of variances within each environment (Mather, 1949). When population variances are related to the mean, a square root or log transformation may result in homogeneity of variances (Bartlett, 1947). Complex epistatic models may be the result of inappropriate scaling, and rescaling may lead to a simpler model. This can be tested quickly with Mather's (1949) scaling tests within individual environments. If all scaling tests are significant and have the same sign, improvement by rescaling (log or antilog) is likely. Rescaling involves transformation of individual scores in each population. Recalculation of parameters after transformation can be performed quickly using the available spreadsheets.

ENVSEG.WK1 is an expanded version of ENVNOSEG.WK1, programmed to include segregating generations in the analysis. ENVSEG.WK1 takes the same input values as ENVNOSEG.WK1 for each population (Figure E-2). The program starts with a joint scaling test on an additive-dominance model without interactions. If the X^2 statistic is not significant at 8 degrees of freedom, the additive-dominance model accurately describes the situation and no further analysis is needed. Estimates and standard errors of parameters are given, and Student's t test can be used to test significance (Figure E-2). Significance of the X^2 statistic indicates inadequacy of this model and the program continues with a joint scaling test including m, [d], [h], e_1, Gde_1, and Ghe_1, as well as a joint scaling test including m [d], [h], e_1, and the epistatic effects [i], [j], and [l]. In both joint scaling tests the X^2 statistic is provided to determine goodness of fit of the model, and if the model is adequate, parameter estimates can be used and their significance tested with Student's t test (Figure E-2). If none of the above models is appropriate, perfect fit estimates and their variances can be obtained to test significance of all parameters in the full model using a t′ test (Figure E-2).

If genotype by environment interaction is significant, analysis per environment is needed and can easily be done using JNTSCALE.WK1 (Ng, 1990). The error variances of estimated genetic parameters in the joint scaling test in JNTSCALE.WK1 are incorrect by a factor of $\sqrt{X^2/(n-k)}$. JNTSCALE.WK1 can also be used to estimate heritabilities in each environment if conditions for these analyses are met (Ng, 1990). The number of effective factors (k) in each environment can be estimated by combining the formulas reported by Mather and Jinks (1971) and Warner (1952) in the formula I below:

$$k = \frac{(\bar{P}_1 - \bar{P}_2)^2}{16\cdot \mathrm{Var}(F2) - 8\cdot \mathrm{Var}(BCP1) - 8\cdot \mathrm{Var}(BCP2)} \quad (I).$$

Unlike Wright's (1934) formula (which is used in JNTSCALE.WK1), formula I does not underestimate the number of effective factors in the presence of dominance. The assumptions for estimating the number of effective factors are equal gene effects, isodirectional distribution of alleles in the parents, and absence of linkage and epistasis. If epistatic effects are insignificant, partitioning of variance components is possible within each environment, assuming the absence of linkage (Mather and Jinks, 1977).

ENVSEG.WK1 calculates standard errors of parameter estimates in the joint scaling test according to Rowe and Alexander (1980). An assumption for the joint scaling test is that the population of sample means for each generation is normally distributed. This condition is met for nonsegregating populations that are assumed to have a normal distribution. In segregating generations, the underlying distribution of BCP1, BCP2, and F2 individuals cannot be assumed normal and the population of means for independent samples is normally distributed only when the sample size is sufficiently large ($n > 100$). Thus, the joint scaling test is only valid if the BCP1, BCP2, and F2 generations are sufficiently large and only then can significance of the parameters be tested with Student's t test with $n - k$ degrees of freedom. Perfect fit estimates in the full model are tested with a t' test and "effective degrees of freedom" are calculated using the procedure of Satterthwaite (1946).

Generation Means Analysis Over More Environments

The generation means analysis can be expanded over many more environments and the differences between environments can be expressed in $e_1 .. e_{m-1}$ orthogonal comparisons (Mather and Jones, 1958), where m is the number of environments. The full nonepistatic model includes m - 1 environmental parameters and 2·(m - 1) G's, representing the interaction of $e_1 .. e_{m-1}$ with genetic parameters [d] and [h]. The coefficients for the interaction parameters can be obtained by multiplying the coefficients of the individual parameters (Mather and Jones, 1958). Joint scaling tests can be used to test significance of interaction of genetic parameters with each of the environments. The easiest way to obtain estimates for the parameters in a full nonepistatic model is to grow parental and F1 generations in each environment. The perfect fit estimates for the parameters in the full model can be obtained from the 3·m generation means as follows:

$$\hat{M} = X^{-1} \cdot Y \qquad \text{(II)}$$

with $\hat{M}$ = Vector of parameters to be estimated,

X = Matrix of expected generation means in terms of parameters in the genetic model,

Y = Vector of generation means.

Variances of the perfect fit estimates can be calculated by summing the variances for individual generation means and squaring their coefficients. Significance can be tested with t' tests. Adequacy of the full nonepistatic model can be tested with a joint scaling test if one or more additional (segregating) generations are grown.

The full digenic interaction model includes 5·(m - 1) G's, representing the interaction of e_1 .. e_{m-1} with [d], [h], [i], [j], and [l]. To estimate all 6·m parameters in the full model, parental, F1, F2, and backcross generations need to be grown in each environment. Perfect fit estimates can be obtained from the 6·m generation means using formula II. Joint scaling tests can be conducted on models with less than 6·m parameters, and on the full digenic interaction model if additional generations are grown that add information to the genetic model (e.g. second backcross or F3 generations).

In the models mentioned above, the joint scaling tests are involved and ENVNOSEG.WK1 and ENVSEG.WK1 do not accommodate data over more than two environments. In these cases, SAS can be used to perform a weighted multiple linear regression analysis through the origin in which least squares estimates are obtained for genetic and environmental parameters, using the coefficients in the genetic model as independent variables and generation means as dependent variable (Figure E-3). The input variables are generation means, weights, and coefficients for the parameters in the model. The output gives an estimate and standard error for each parameter and a t test for significance (Figure E-3). Note that this t test is only valid when sufficiently large samples ($n > 100$) are obtained from segregating generations. For each generation, the observed and predicted means are given and the weighted sum of squared residuals corresponds with the X^2 in the joint scaling test (Rowe and Alexander, 1980). The standard errors in Figure E-3 show a discrepancy of $\sqrt{X^2 / (n - k)} = 0.894$ with those mentioned for the same data set in Mather and Jinks (1971). SAS can be used to easily conduct joint scaling tests other than those in JNTSCALE.WK1, ENVNOSEG.WK1, and ENVSEG.WK1. Furthermore, SAS can be used to conduct joint scaling tests for adequacy of a full model by addition of generation means to the ones needed for fitting the full model. The package of microcomputer software reported in this paper greatly facilitates cumbersome calculations involved with generation means analysis over environments and reduces the chance for errors.

```
Generation means analysis of nonsegregating generations over two environments

Generation  Environment   Mean       n        @ var    Variance  St. dev.  Var (x)   St. err.
-------------------------------------------------------------------------------------------
Parent 1         1       144.59     240  1057.054  1061.477     32.58     4.423     2.103
F1               1       155.32     240   631.106   633.747     25.17     2.641     1.625
Parent 2         1       107.45     240  1680.731  1687.764     41.08     7.032     2.652
Parent 1         2        79.20     480  5899.015  5911.330     76.89    12.315     3.509
F1               2        94.70     480  1758.444  1762.115     41.98     3.671     1.916
Parent 2         2        66.65     480  9193.858  9213.052     95.98    19.194     4.381

            PRESS '<ALT> R' TO RECALCULATE SPREAOSHEET


Estimates of parameters in additive-dominance model without interaction
---------------------------------------------------------------------------
                          St. err.      t
                         --------------------      Note: Significance of each
    m  =       98.1665    4.2307   23.2034               parameter is tested
   [d] =       15.2861    4.0310    3.7922               using the t statistic
   [h] =       26.9934    5.3694    5.0273               with 2 degrees of
    e  =       29.3917    2.7470   10.6994               freedom
---------------------------------------------------------------------------

Testing the adequacy of the additive-dominance model without interaction:
--------------------------------------------------------------------------------
Generation  Environment   Obs. x   Expt. x  Squared Oeviation  Goodness of Fit
----------  -----------   -------  -------  -----------------  ---------------
Parent 1         1        144.59   142.84     3.0475               0.689
F1               1        155.32   154.55     0.5904               0.224
Parent 2         1        107.45   112.27    23.2526               3.307
Parent 1         2         79.20    84.06    23.6287               1.919
F1               2         94.70    95.77     1.1412               0.311
Parent 2         2         66.65    53.49   173.2186               9.025
                                                               ---------------
                                  Sum of Contributions =          15.473
--------------------------------------------------------------------------------
                          Note: the sum of contributions can be checked via
                          a chi-square table for (6-4=2) degrees of freedom.


Estimates of the additive, dominance and interaction parameters in full model
-------------------------------------------------------------------------------------------
                                             Effective
                          St. err.      t'       df     Variance
                         ----------  -------  -------  ---------
    m  =       99.473      1.639     60.70   1342.98    2.68527     Note: Significance
   [d] =       12.423      1.639      7.58   1342.98    2.68527           of each parameter
   [h] =       25.537      2.065     12.37   2030.46    4.26319           is tested using
    e  =       26.548      1.639     16.20   1342.98    2.68527           the t' statistic.
   Gde =        6.148      1.639      3.75   1342.98    2.68527
   Ghe =        3.762      2.065      1.82   2030.46    4.26319
-------------------------------------------------------------------------------------------
    m  =    midparent value                                 = 0.25(P1.1+P2.1+P1.2+P2.2)
   [d] =    difference of AA & aa from midparent value      = 0.25(P1.1-P2.1+P1.2-P2.2)
   [h] =    difference of Aa from midparent value           =
                                              0.5(F1.1+F1.2)-0.25(P1.1+P2.1+P1.2+P2.2)
    e  =    difference of env. from mean; +e for 1; -e for 2  = 0.25(P1.1+P2.1-P1.2-P2.2)
   Gde =    interaction between [d] and e                   = 0.25(P1.1-P2.1-P1.2+P2.2)
   Ghe =    interaction between [h] and e                   =
                                              0.5(F1.1-F1.2)-0.25(P1.1+P2.1-P1.2-P2.2)
```

Figure E-1. Output of ENVNOSEG.WK1 with data on plant spread from Powers (1941), the environmental parameter is -e for 1938 (environment 2) and +e for 1939 (environment 1).

```
Generation means analysis including segregating generations over two environments

Generation  Environment  Mean         n       @ var     Variance  St. dev.  Var (x)  St. error
----------- ---------- ---------  -------- ---------- --------  --------  -------  ---------
Parent 1         1       144.59      240  1057.054  1061.477     32.58     4.423      2.103
8CP1             1       153.49      240   952.191   956.175     30.92     3.984      1.996
F1               1       155.32      240   631.106   633.747     25.17     2.641      1.625
F2               1       139.65      240  1648.276  1655.172     40.68     6.897      2.626
8CP2             1       130.46      240   897.484   901.239     30.02     3.755      1.938
Parent 2         1       107.45      240  1680.731  1687.764     41.08     7.032      2.652
Parent 1         2        79.20      480  5899.015  5911.330     76.89    12.315      3.509
8CP1             2        88.15      960  6806.246  6813.343     82.54     7.097      2.664
F1               2        94.70      480  9193.858  9213.052     95.98    19.194      4.381
F2               2        84.25      960  5912.454  5918.619     76.93     6.165      2.483
8CP2             2        77.05      960  4823.944  4828.974     69.49     5.030      2.243
Parent 2         2        66.65      480  1758.444  1762.115     41.98     3.671      1.916

               PRESS '<ALT> R' TO RECALCULATE SPREADSHEET


Estimates in additive-dominance model without interactions
-------------------------------------------------------------------------
                          St. err.     t
                       --------------------     Note: Significance of each
  m  =      101.2355    2.5266   40.0685              parameter is tested
 [d] =       13.7581    2.5304    5.4372              using the t statistic
 [h] =       24.3538    4.6239    5.2669              with 8 degrees of
  e  =       27.8860    1.6820   16.5792              freedom
-------------------------------------------------------------------------

Testing the adequacy of the additive-dominance model without interactions
-------------------------------------------------------------------------------
Generation  Environment   Obs. x    Expt. x  Squared Deviation  Goodness of Fit
----------  -----------   -------   -------  -----------------  ---------------
P1               1        144.59    142.88     2.9252                0.661
8CP1             1        153.49    148.18    28.2224                7.084
F1               1        155.32    153.48     3.4027                1.289
F2               1        139.65    141.30     2.7174                0.394
8CP2             1        130.46    134.42    15.6767                4.175
P2               1        107.45    115.36    62.6218                8.905
P1               2         79.20     87.11    62.5311                5.078
8CP1             2         88.15     92.41    18.1093                2.552
F1               2         94.70     97.70     9.0201                0.470
F2               2         84.25     85.53     1.6293                0.264
BCP2             2         77.05     78.65     2.5516                0.507
P2               2         66.65     59.59    49.8242               13.572
                                                                ---------------
                                   Sum of Contributions =               44.950
-------------------------------------------------------------------------------
                        Note: the sum of contributions can be checked via
                        a chi-square table for (12-4=8) degrees of freedom.
```

Figure E-2. Output of ENVSEG.WK1 with data on plant spread from Powers (1941), the environmental parameter is -e for 1938 (environment 2) and +e for 1939 (environment 1).

```
Estimates in additive-dominance model with environment interaction
-----------------------------------------------------------------------
                             St. err.      t
                          --------------------      Note: Significance
    m  =        99.7534    1.0419     95.7440             of each parameter
   [d] =        13.5545    1.0013     13.5367             is tested using
   [h] =        24.6923    2.0158     12.2495             the t statistic
    e  =        26.4340    1.0419     25.3715             with 6 degrees of
   Gde =         6.1308    1.0013      6.1227             freedom
   Ghe =         4.8501    2.0158      2.4061
-----------------------------------------------------------------------

Testing the adequacy of the additive-dominance model with environment interaction
------------------------------------------------------------------------------
Generation  Environment   Obs. x    Expt. x  Squared Deviation  Goodness of Fit
----------  -----------  -------    -------  -----------------  ---------------
P1              1         144.59     145.87    1.6451                0.372
BCP1            1         153.49     150.80    7.2294                1.815
F1              1         155.32     155.73    0.1680                0.064
F2              1         139.65     140.96    1.7125                0.248
BCP2            1         130.46     131.12    0.4303                0.115
P2              1         107.45     106.50    0.8984                0.128
P1              2          79.20      80.74    2.3813                0.193
BCP1            2          88.15      86.95    1.4342                0.202
F1              2          94.70      93.16    2.3665                0.123
F2              2          84.25      83.24    1.0190                0.165
BCP2            2          77.05      79.53    6.1440                1.221
P2              2          66.65      65.90    0.5689                0.155
                                                                ---------------
                                   Sum of Contributions =            4.801
------------------------------------------------------------------------------
                        Note: the sum of contributions can be checked via
                        a chi-square table for (12-6=6) degrees of freedom.

Estimates in model with nonallelic interactions
---------------------------------------------------------------------
                             St. err.      t
                          --------------------
    m  =        98.0907   23.0413      4.2572       Note: Significance
   [d] =        12.3956    3.3035      3.7523             of each parameter
   [h] =        26.6850   53.6462      0.4974             is tested using
   [i] =         3.6940   22.8297      0.1618             the t statistic
   [j] =        12.3798   13.3946      0.9242             with 5 degrees of
   [l] =         2.1005   32.2079      0.0652             freedom
    e  =        27.8484    1.9494     14.2858
---------------------------------------------------------------------
```

Figure E-2. Continued.

```
Testing the adequacy of the model with nonallelic interactions
-------------------------------------------------------------------------------
Generation  Environment   Obs. x    Expt. x  Squared Deviation  Goodness of Fit
----------  -----------  -------    -------  -----------------  ---------------
P1               1        144.59    142.03    6.5602                1.483
BCP1             1        153.49    150.02   12.0202                3.017
F1               1        155.32    154.72    0.3545                0.134
F2               1        139.65    139.81    0.0246                0.004
BCP2             1        130.46    131.44    0.9554                0.254
P2               1        107.45    117.24   95.7951               13.622
P1               2         79.20     86.33   50.8637                4.130
BCP1             2         88.15     94.33   38.1449                5.375
F1               2         94.70     99.03   18.7296                0.976
F2               2         84.25     84.11    0.0196                0.003
BCP2             2         77.05     75.74    1.7144                0.341
P2               2         66.65     61.54   26.1053                7.111
                                                                ---------------
                                   Sum of Contributions =          36.450
-------------------------------------------------------------------------------
                                 Note: the sum of contributions can be checked via
                                 a chi-square table for (12-7=5) degrees of freedom.
```

```
Estimates of the genetic parameters and their interaction with environment in full model
-----------------------------------------------------------------------------------------
                                               Effective
                           St. err.     t'        df     Variance
                          ---------  ---------  --------- ---------
   m  =       98.1225      8.5924    11.4197    1354.19   73.8289
  [d] =       12.4225      1.3096     9.4856    1188.63    1.7151
  [h] =       28.4225     20.2159     1.4059    2144.25  408.6827      Note: Significance
  [i] =        1.3500      8.4920     0.1590    1292.80   72.1138            of each parameter
  [j] =        9.2850      5.1698     1.7960    2928.57   26.7270            is tested using
  [l] =       -1.5350     12.6652    -0.1212    3246.68  160.4085            the t' statistic.
   e  =       18.5975      8.5924     2.1644    1354.19   73.8289
  Gde =        6.1475      1.3096     4.6941    1188.63    1.7151
  Ghe =       24.6975     20.2159     1.2217    2144.25  408.6827
  Gie =        7.9500      8.4920     0.9362    1292.80   72.1138
  Gje =       -0.3650      5.1698    -0.0706    2928.57   26.7270
  Gle =      -12.9850     12.6652    -1.0252    3246.68  160.4085
-----------------------------------------------------------------------------------------
 m = 0.25(P1.1+P2.1+P1.2+P2.2)+2(F2.1+F2.2)-BCP1.1-BCP2.1-BCP1.2-BCP2.2
[d]= 0.25(P1.1-P2.1+P1.2-P2.2)
[h]= -0.75(P1.1+P2.1+P1.2+P2.2)-0.5(F1.1+F1.2)-4(F2.1+F2.2)+3(BCP1.1+BCP2.1+BCP1.2+BCP2.2)
[i]= BCP1.1+BCP2.1+BCP1.2+BCP2.2-2(F2.1+F2.2)
[j]= -0.5(P1.1-P2.1+P1.2-P2.2)+BCP1.1-BCP2.1+BCP1.2-BCP2.2)
[l]= 0.5(P1.1+P2.1+P1.2+P2.2)+F1.1+F1.2+2(F2.1+F2.2)-2(BCP1.1+BCP2.1+BCP1.2+BCP2.2)
 e = 0.25(P1.1+P2.1-P1.2-P2.2)+2(F2.1-F2.2)-BCP1.1-BCP2.1+BCP1.2+BCP2.2
Gde= 0.25(P1.1-P2.1-P1.2+P2.2)
Ghe= -0.75(P1.1+P2.1-P1.2-P2.2)-0.5(F1.1-F1.2)-4(F2.1-F2.2)+3(BCP1.1+BCP2.1-BCP1.2-BCP2.2)
Gie= BCP1.1+BCP2.1-BCP1.2-BCP2.2-2(F2.1-F2.2)
Gje= -0.5(P1.1-P2.1-P1.2+P2.2)+BCP1.1-BCP2.1-BCP1.2+BCP2.2
Gle = 0.5(P1.1+P2.1-P1.2-P2.2)+(F1.1-F1.2)+2(F2.1-F2.2)-2(BCP1.1+BCP2.1-BCP1.2-BCP2.2)
```

Figure E-2. Continued.

```
data gma;
  input mean weight m d h e Gde Ghe;
  cards;
  144.59   0.2261    1    1     0    1    1     0
  153.49   0.2510    1    0.5   0.5  1    0.5   0.5
  155.32   0.3787    1    0     1    1    0     1
  139.65   0.1450    1    0     0.5  1    0     0.5
  130.46   0.2663    1   -0.5   0.5  1   -0.5   0.5
  107.45   0.1422    1   -1     0    1   -1     0
   79.20   0.0812    1    1     0   -1   -1     0
   88.15   0.1409    1    0.5   0.5 -1   -0.5  -0.5
   94.70   0.0521    1    0     1   -1    0    -1
   84.25   0.1622    1    0     0.5 -1    0    -0.5
   77.05   0.1988    1   -0.5   0.5 -1    0.5  -0.5
   66.65   0.2724    1   -1     0   -1    1     0
proc glm;
  weight weight;
  model mean=m d h e Gde Ghe/noint p;
run;
```

```
Oependent Variable: MEAN
Weight:             WEIGHT
                                      Sum of              Mean
Source                     OF        Squares            Square   F Value      Pr > F

Model                       6     34386.29954       5731.04992   7161.96      0.0001
Error                       6         4.80124          0.80021
Uncorrected Total          12     34391.10078

Source                     OF     Type III SS      Mean Square   F Value      Pr > F

M                           1     7335.422719      7335.422719   9166.91      0.0001
0                           1      146.631813       146.631813    183.24      0.0001
H                           1      120.071018       120.071018    150.05      0.0001
E                           1      515.102971       515.102971    643.71      0.0001
GOE                         1       29.998146        29.998146     37.49      0.0009
GHE                         1        4.632579         4.632579      5.79      0.0529

                                                T for H0:     Pr > |T|    Std Error of
Parameter                    Estimate         Parameter=0                   Estimate

M                         99.75342266               95.74       0.0001     1.04187649
0                         13.55446310               13.54       0.0001     1.00131154
H                         24.69233829               12.25       0.0001     2.01578462
E                         26.43396192               25.37       0.0001     1.04187649
GOE                        6.13077561                6.12       0.0009     1.00131154
GHE                        4.85014317                2.41       0.0529     2.01578462

Observation             Observed            Predicted               Residual
                         Value                Value

      1              144.59000000         145.87262330           -1.28262330
      2               79.20000000          80.74314823           -1.54314823
      3              107.45000000         106.50214586            0.94785414
      4               66.65000000          65.89577325            0.75422675
      5              155.32000000         155.72986604           -0.40986604
      6               94.70000000          93.16165586            1.53834414
      7              139.65000000         140.95862531           -1.30862531
      8               84.25000000          83.24055830            1.00944170
      9              153.49000000         150.80124467            2.68875533
     10               88.15000000          86.95240205            1.19759795
     11              130.46000000         131.11600595           -0.65600595
     12               77.05000000          79.52871456           -2.47871456
            Sum of Squared Residuals                              4.80123828
            Sum of Squared Residuals - Error SS                  -0.00000000
```

Figure E-3. SAS program and output for weighted linear regression using data on plant spread from Powers (1941), the environmental parameter is -e for 1938 and +e for 1939.

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BIOGRAPHICAL SKETCH

Jan Barten was born on 3 August 1963 to Dini and Peter Barten - van Duynhoven in Oploo, The Netherlands. He went to the 'Elzendaal College' in Boxmeer, The Netherlands, and graduated in May 1981. He continued his education at the Agricultural University in Wageningen, The Netherlands, where he developed an interest in genetics and plant breeding. He received his 'Agricultural Engineer' degree in November, 1987, with a major in plant breeding and minors in plant physiology, horticulture and computer science. Research projects involved improvement of selection methodology in potato breeding, investigation of the physiological backgrounds of scenescence in pollen, and nondestructive growth analyses in tomato. In January, 1988, he received a research assistantship at the University of Florida to study blossom-end scarring in tomato, leading to the findings reported in this dissertation.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J. W. Scott

John W. Scott, Chair
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Mark J. Bassett

Mark J. Bassett
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas E. Humphreys

Thomas E. Humphreys
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Rebecca L. Darnell
Assistant Professor of
Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

A. Khuri

Andrawus I. Khuri
Professor of Statistics

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1991

Jack L. Fry

Dean, College of Agriculture

Madelyn Lockhart

Dean, Graduate School